(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 August 2002 (22.08.2002)

PCT

(10) International Publication Number
WO 02/064820 A1

(51) International Patent Classification[7]: C12Q 1/68, C12N 15/11

(21) International Application Number: PCT/AU02/00122

(22) International Filing Date: 8 February 2002 (08.02.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PR 2975 9 February 2001 (09.02.2001) AU

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/064820 A1

(54) Title: DNA MARKERS FOR MEAT TENDERNESS

(57) Abstract: A method for assessing the tenderness of meat from an animal, comprising the step of testing the animal for the presence or absence of a genetic marker selected from the group consisting of: (1) an allele of the gene encoding calpastatin (CAST) associated with peak-force variation or genetic variation located other than in the CAST gene which shows allelic association with the CAST allele; and (2) an allele of the gene encoding lysyl oxidase (LOX) associated with intron compression of the semitendinous muscle or genetic variation located other than in the LOX gene which shows allelic association with the LOX allele.

DNA MARKERS FOR MEAT TENDERNESS

Technical Field

The present invention is concerned with genetic markers for meat tenderness in animals, and with methods and oligonucleotide probes for assessing meat tenderness in said animals, and a kit for this purpose. The invention is useful for the selection of animals which show desirable traits in meat tenderness either for breeding or to select animals destined to be slaughtered for food.

Background Art

Meat tenderness is an important issue for consumers, and one which can influence demand sufficiently for an especially tender meat to command a premium price in the marketplace. The physiological change in muscle structure during the postmortem period is complex but clearly seems to be at least one factor in meat tenderness. The calpain/calpastatin system is an endogenous, calcium-dependent proteinase system, theorised to initiate *in vivo* muscle protein degradation. Calpastatin appears to inhibit calpain activity and therefore may be assumed to have a role in meat tenderness through the regulation of postmortem proteolysis. In particular, calpain is response for the breakdown of myofibril protein, which is closely related to meat tenderness.

It might therefore be suspected that calpastatin activity will be related to meat tenderness. Indeed, an increase in postmortem calpastatin activity has been correlated to reduced meat tenderness. Nevertheless, despite such observations, no clear link between the CAST gene, which encodes calpastatin, and meat tenderness has been established.

For example, Lonergan et al. (1995) undertook a restriction fragment length polymorphism analysis at CAST and failed to find an association with either calpastatin

activity or tenderness in cross bred offspring of sires from eight breeds. Chung et al. (1999) measured calpastatin activity, Warner-Bratzler Shear Force and myofibril fragmentation index in forty-seven purebred Angus bulls. However, they concluded that "PCR single-strand conformation polymorphism analysis of the calpastatin gene was not useful for prediction of calpastatin activity, myofibril fragmentation index or meat tenderness".

It is long known that one of the actions of lysyl oxidase (LOX) is to initiate crosslink formation at an early stage in collagen fibrillogenesis (e.g., Cronlund *et al.*, 1985). The action of lysyl oxidase is intensively studied with hundreds of publications on a variety of aspects of its importance in cancer (Giampuzzi *et al.*, 2001), the vasculature (Nellaiappan *et al.*) and other tissue and organ systems.

Variation at the gene itself has not been associated with differences in beef tenderness although LOX has always been seen as a strong candidate on biochemical grounds for a gene contributing to the collagen component of tenderness. Analysis of genetic linkage has implicated the genomic region that includes LOX in linkage analysis of family variation in adhesion and instron compression of the semitendinosis muscle (STADH and STIC; Drinkwater *et al.*, 1999). However, LOX itself has not been associated with these measures of tenderness through the study of population associations.

Meat tenderness is a complicated trait because there are many sources of variation that affect postmortem meat tenderisation. Some of these are non-genetic effects such as the age of the beast, the nature of its feed, degree of stress prior to slaughter, carcass chilling, postmortem ageing time and cooking and testing methods. It has been suggested (e.g. Koohmaraie (1994)) that approximately 30% of the variation in tenderness in meat can be explained by additive gene effects within a single

breed, and that approximately 70% of the variation is explained by environmental and non-additive gene effects. In the Lonergan study the cattle were slaughtered at just over 1 year of age (430 days), the sample contained only 83 animals of random peak-force values, and the sample consisted entirely of crosses between various taurine breeds. Likewise, in the Chung study purebred Angus bulls only 280 days of age were used. In addition, in neither study were the animals selected for extreme peak-force values, and it therefore seems that environmental and non-fixed genetic effects may have contributed to the failure to identify any genetic linkage between the CAST gene and meat tenderness.

Summary of the Invention *[Revise this once claims are settled]*

Through using a protocol designed to reduce the influence of fixed genetic and environmental effects, the present inventor was unexpectedly able to show allelic association between the CAST and LOX genes and meat tenderness. In general terms, therefore, the present invention is concerned with genetic markers for meat tenderness in animals killed for meat which are polymorphisms of the CAST and LOX genes or polymorphisms which show allelic association therewith.

Accordingly, in a first aspect of the present invention there is provided a method for assessing the tenderness of meat from an animal, comprising the step of testing the animal for the presence or absence of a genetic marker selected from the group consisting of:

(1) an allele of the gene encoding calpastatin (CAST) associated with peak-force variation or genetic variation located other than in the CAST gene which shows allelic association with the CAST allele; and

(2) an allele of the gene encoding lysyl oxidase (LOX) associated with variation in instron

compression of the semitendinosis muscle or genetic variation located other than in the LOX gene which shows allelic association with the LOX allele.

Preferably, the allele tested for is located in the 3' UTR of CAST, and is typically CAST3 D/E allele 1, having the following partial DNA sequence:

catttggaaaacgatgcctcacgtgttcttcagtgttctgatttctcat gacccctttcctcttGgacttgtgggactgtgtttgatgtttccctgggttgttgttt ataagtcagtcataaAatactgtgcattgggcacatgtctcctcttgagctgctaatc gtaga (SEQ ID NO:1),

CAST3 D/E allele 2, having the following partial DNA sequence:

catttggaaaacgatgcctcacgtgttcttcagtgttctgatttctcat gacccctttcctcttAgacttgtgggactgtgtttgatgtttccctgggttgttgttt ataagtcagtcataaAatactgtgcattgggcacatgtctcctcttgagctgctaatc gtaga (SEQ ID NO:2)

or CAST3 D/E allele 3, having the following partial DNA sequence:

catttggaaaacgatgcctcacgtgttcttcagtgttctgatttctcat gacccctttcctcttAgacttgtgggactgtgtttgatgtttccctgggttgttgttt ataagtcagtcataaTatactgtgcattgggcacatgtctcctcttgagctgctaatc gtaga (SEQ ID NO:3).

Reduced meat toughness is selected for by rejecting animals with the "11" and "12" genotypes and accepting animals with the "22" or "23" genotypes. In the sequences given above, the allelic difference is highlighted with a capital letter. These three alleles in the D/E DNA fragment are due to two SNP (single nucleotide polymorphisms). The first SNP is at base 2655 of Genbank sequence L14450, which is the same as base 2959 of Genbank sequence AF159246; it is a G to A change so that allele 1 has G and alleles 2 and 3 have A. The second SNP is an A to T change 58 base pairs 3' to the first SNP. Since only three alleles have been noted for this region, with 2 SNPs, it implies that there is complete linkage disequillibrium between allele 2 and allele 3, and allele

3 may have evolved from allele 2. This is expected since they are 58 base pairs apart. For predictive purposes, a test of the second SNP which gives a result of allele 3 is equivalent to a test of the first SNP giving a result of allele 2. This is consistent with the peak force values of animals that are '23' heterozygotes, all of whom have low peak force values. While not wishing to be bound by theory, it is believed that these polymorphisms are linked to a mutation in or near the calpastatin gene (perhaps in the promoter or an intron) which results in reduced calpastatin expression or activity.

A further polymorphism has been identified in the 5' UTR of the CAST gene and other polymorphisms which exhibit allelic association with the polymorphism of the 3' UTR, and therefore also act as genetic markers for the tenderness characteristics described above, may also be present at least within the genomic DNA embracing the coding region of the CAST gene and the 5' UTR and 3' UTR regions of that gene. In addition, where there has been a recent reduction in population size for a species, particular haplotypes of individuals will be relatively over-represented. If insufficient time has elapsed to cause allelic association to decay, there will be linkage disequilibrium even for alleles which are far apart. Livestock species such as cattle have been domesticated from a relatively small pool of wild ancestors in recent times, and therefore in these species allelic association is found between alleles that may be remote physically. Thus, it may be expected that regions of genetic variation that are outside the CAST gene will also show allelic association with the polymorphisms in the CAST gene described above, and therefore will be suitable genetic markers for the characteristic of peak-force variation. Hence, these polymorphisms may also be used to assess meat tenderness.

In particular the CAST5 microsatellite polymorphism (Nonneman *et al*, 1999) has been found to be

useful as a genetic marker for meat tenderness. Of the more common alleles, alleles 7 and 9 have been found to be associated with low peak-force and allele 3 to be associated with high peak-force.

Therefore, the invention encompasses, in preferred embodiments, the further step of testing for the presence or absence of one or more additional genetic markers such as alleles of the gene encoding calpastatin associated with peak-force variation, in particular, with testing for the presence or absence of CAST5 allele 7 or 9 and/or the presence or absence of CAST5 allele 3. The most favorable results when the presence of CAST D/E allele 2 has been established is to have CAST5 allele 7 or allele 9 present also, whereas the benefits of the presence of CAST D/E allele 2 are to some degree counteracted if the animal also possesses CAST5 allele 3.

The LOX polymorphism has also been shown to be a genetic marker for meat tenderness, and allele 1 or allele 2 may be tested for. Just as for the CAST gene, allelic association may be exhibited to alleles located outside the LOX gene.

According to a second aspect of the present invention, there is provided a genetic marker for meat tenderness in an animal which is a polymorphic form of the CAST gene, being the CAST3 D/E polymorphism or the LOX polymorphism.

According to a third aspect of the present invention there is provided an isolated DNA molecule comprising the nucleotide sequence set forth in SEQ ID NO:1, SEQ ID NO:2 or SEQ ID NO:3.

According to a fourth aspect of the present invention there is provided an isolated DNA molecule consisting of the nucleotide sequence set forth in SEQ ID NO:1, SEQ ID NO:2 or SEQ ID NO:3.

According to a fifth aspect of the present invention there is provided a method for selecting an animal likely to yield meat of improved tenderness, comprising the steps

of:

(1) testing the animal for the presence of an allele of the gene encoding calpastatin (CAST) associated with low peak-force or genetic variation located other than in the CAST gene which shows allelic association with the CAST allele and/or for the presence of an allele of the LOX gene associated with the low instron compression of the semitendinosis muscle or genetic variation located other than in the LOX gene which shows allelic association with the LOX allele; and

(2) selecting animals which have the CAST and/or LOX allele and/or genetic variation in allelic association therewith.

Advantageously, in order to assess the tenderness of meat from an animal and/or select an animal likely to yield meat of improved tenderness testing may comprise the steps of:

(1) obtaining a biological sample from the animal;

(2) extracting DNA from the sample;

(3) amplifying DNA from the CAST or LOX gene and/or from regions of genetic variation which show allelic association to polymorphisms of the relevant one of the CAST or LOX gene; and

(4) identifying the allele present in the amplified DNA.

Typically the allele identified in step (4) is one of CAST3 D/E allele 1, CAST3 D/E allele 2 and CAST3 D/E allele 3 described above and/or the CAST5 alleles described above.

Preferably the biological sample is blood, but other biological samples from which DNA can be amplified may be used. For example, hair root samples, cheek scrapings, skin samples and the like may be used.

Typically amplification is performed using the polymerase chain reaction (PCR), but other DNA

amplification methods such as the ligase chain reaction are well known in the art, and may alternatively be used.

Preferably the alleles are identified by polyacrylamide gel electrophoresis techniques such as SSCP, or by other techniques well known to the person skilled in the art such as RFLP analysis.

In a sixth aspect the invention provides an oligonucleotide probe for amplification of a genetic marker associated with peak-force variation, said genetic marker being either an allele of the gene encoding calpastatin (CAST) or genetic variation located other than in the CAST gene which shows allelic association with said allele.

Typically the probe is selected from the group consisting of:

castd 5′ cat ttg gaa aac gat gcc tca c 3′

caste 5′ tct acg att agc agc tca aga gga g 3′

CAST5U1 5′-GTAAAGCCGCACAAAACACACCCAGG-3′

CAST5D1 5′-GTTTCTGGACCCTCTGGATGAGGAAGCGG-3′.

In view of the designation of the primers as CASTD and CASTE, the amplified fragment of the CAST gene is referred to from time to time as the CAST D/E fragment and the polymorphism as the CAST D/E polymorphism.

According to a seventh aspect of the present invention there is provided an oligonucleotide probe for amplification of a genetic marker associated with variation in instron compression of the semitendinosis muscle, the genetic marker being either an allele of the gene encoding lysyl oxidase (LOX) or genetic variation located other than in the LOX gene which shows allelic association with said allele.

Typically the oligonucleotide probe is an oligonucleotide probe selected from the group consisting of:

LOX K5: 5′ tat cac tga tgt caa acc tg 3′

LOX K6: 5′ act cag gca cca aat agc tg 3′

According to an eighth aspect of the present invention there is provided a kit for use in assessing the tenderness of meat from an animal and/or selecting an animal likely to yield meat of improved tenderness, comprising oligonucleotide probes for amplification of at least one genetic marker for meat tenderness, said genetic marker being either an allele of the gene encoding calpastatin (CAST) or genetic variation located other than in the CAST gene which shows allelic association with said allele, or an allele of the LOX gene associated with low instron compression of the semitendinosis muscle or genetic variation located other than in the LOX gene which shows allelic association with the LOX allele, and means for amplifying DNA.

The primers used to amplify the DNA are the CASTD and CASTE primers and/or the CAST5U1 and CAST5D1 primers for amplifying the CAST5 polymorphism. However, other primers able to amplify polymorphisms associated with a reduction in toughness in meat are envisaged, whether these be primers which amplify a polymorphism other than the CAST3 D/E polymorphism or CAST5 polymorphism, or other primers able to amplify the CAST3 D/E fragment of CAST5 polymorphism.

The methods of the invention may be used both for the selection of breeding animals and for the selection of unpedigreed animals for entry into feed lots. In the latter case, the methods of the invention allow for animals with unsuitable pedigrees to be excluded from feed lots on the basis that highly tender meat is unlikely to be attained with these animals even after a long feed lot holdings. Alternatively, such measurements may allow for determination of the optimum time to reach maximum meat tenderness. The invention is therefore also concerned with animals when selected by the method of the invention, their progeny and the use of both selected animals and their progeny for breeding, as well as meat from these animals.

The methods of the invention are applicable to animals including but not limited to cattle and other bovids, including water buffalo and bison, to other ungulates, including sheep, goats and deer, and pigs and chickens.

Throughout this specification, the words "comprise", "comprises" and "comprising" are used in a non-exclusive sense, except where the context requires otherwise.

It will be clearly understood that, although a number of prior art publications are referred to herein, this reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art, in Australia or in any other country.

Brief Description of the Drawings

Preferred embodiments of the present invention will now be described, by way of example only, with reference to the accompanying drawings, in which:

Fig 1 is a photograph of a single strand conformational polymorphism (SSCP) gel which shows genotypes for the CAST3 D/E polymorphism, from left to right, 11, 22, blank, 11, 12, 12, 12, 12, 11, 22.

Fig 2 a & b show the distribution of Warner-Bratzler peak-force measurements in the two samples of 169 and 77 animals respectively. Note that extremes were used so there is no middle to the distribution. It does not imply that the distribution is bi-modal. Note different scales in the figures.

Fig 3 a & b are a plot of the raw Warner-Bratzler peak-force measurements against the CAST genotypes. Note the gap in the middle due to the use of extremes of the distribution. Note the similarity between the distributions in the two samples.

Fig 4 is a boxplot of the residual Warner-Bratzler peak-force measurements (X1) for each genotype

for the first sample. The median quarter and three-quarter percentiles, whiskers and outliers are shown.

Fig 5 shows the distribution of DNA fragment sizes for the CAST5 microsatellite. Horizontal axis is the frequency of each allele and the vertical axis is the DNA fragment size. Alleles are labelled in increasing DNA fragment size so allele m1 in this distribution is less than 132 bp, m2 < 136 bp, m3 < 138 bp, m4 < 142 bp, m5 < 144 bp, m6 < 146 bp, m7 < 148 bp, m8 < 151 bp, m9 < 153 bp, m10 < 155 bp, m11 < 157 bp, m12 < 159 bp, m13 < 161 bp. DNA fragments were not found in some of the 2 bp bins, e.g., in the less than 134 bp bin, and these are either extremely rare or non-existent.

Fig 6 is a box plot of raw LD peak-force values along the horizontal axis versus CAST5 microsatelite allele identity along the vertical axis. The boxes contain the median value, represented by the dot, a box representing the 25 and 75 percentile and whiskers indicating the expected range for the distributions, with outliers indicated by open circles. Care must be taken in interpreting this figure since there are some alleles that are rare, such as m1, m2 and m13 (see Figure 5 for the full distribution, so interpretations made on those alleles are not particularly informative. Note particularly that this is a distribution of extreme values, so the median value will swing from a low to a high value if half the samples are high values.

Figure 7 is a photograph of a single strand conformational polymorphism gel showing the genotypes of LOX from left to right, 11, 11, 12, 12, 22, 11.

Figure 8 shows the distribution of instron compression measurements for the two samples of 166 and 87 animals combined. Note that extremes were used so there is no middle to the distribution. It does not imply that the distribution is bi-modal.

Figure 9 shows the distribution of adhesion measurements for the two samples of 166 and 87 animals

combined. Although the sample was selected for extremes of instron compression, this has not been translated into a series of extreme adhesion measurements.

Figure 10 is a plot of STIC versus STADH for the combined sample. Note the non-uniformity caused by the selected STIC values.

Modes for Performing the Invention

Example 1 - Identification of CAST3 D/E polymorphism

Cattle were chosen from the DNA Bank of the Cattle and Beef Cooperative Research Centre located in Brisbane, Australia to have as diverse a genetic and phenotypic background as possible. Information stored in the CRC Database was used to select animals. Animals of extremes of peak-force were selected, although animals with peak-force measures above 12 were excluded since they might have confounded peak-force measurements. In essence, the procedure was to select cattle in each contemporary group which were of phenotypic extreme measures, to ensure that no sire was represented by a cluster of offspring, that all markets and finishing regimes were included in each extreme, so that extremes were not biased by being representative of a particular market or finishing regime. A total of 169 samples were obtained (Table 1) for the first sample. A second sample of 77 animals (Table 6) were analysed as a check to determine whether the same allelic association could be observed in another sample.

These DNA samples were genotyped for the CAST (calpastatin) D/E DNA fragment using the primers

castd 5′ cat ttg gaa aac gat gcc tca c 3′

caste 5′ tct acg att agc agc tca aga gga g 3′.

The conditions of the polymerase chain reaction (PCR) are an annealing temperature of 60 Celsius, 2.5 mM Magnesium chloride, and reagent mixes obtained from Biotech International. The DNA fragments were labelled via the incorporation of ^{32}P dCTP into the fragments during

the PCR, and the fragments were visualised by autoradiography using X-Ray film exposed overnight at room temperature. Alleles were scored in numerical order where the fastest migrating allele is number 1.

The genotypes were analysed using generalised linear models (GLM) following the equation peak-force = 1 + genotypes nested within fixed effects + error implemented via the S-PLUS software. Fixed effects that were considered were breed, finish (Australia, Korea, Japan), contemporary group (cohort), region (pasture v grain, north v south) and the covariate of final weight. The genotypes were nested within region and breed since pure-bred offspring of taurine sires were not pastured in the north. The size of the effects associated with genotype was estimated by the comparison of variances (eg, Andersson-Eklund and Rendel, 1993). To estimate the size of effect associated with genotypic substitution, the same model was fitted without the calpastatin genotypes. Residuals were extracted and compared to the calpastatin genotypes. These were analysed using an analysis of variance to obtain adjusted means for each genotype Plots of raw and residual peak-force values against calpastatin genotypes were constructed.

Example 2 - Analysis of CAST3 D/E polymorphism

There are two common alleles (Figure 1) and at least one rare allele for the CAST D/E polymorphism and both the common alleles are found in all the breeds, although there are clear differences in genotype frequency within the breeds. Zebu breeds have a greater frequency of the '11' genotype (Tables 2 and 7) than taurine breeds in this sample.

The raw values (Figures 2a & 2b) were then plotted against the CAST genotypes (Figures 3a & 3b) and these associations are sufficiently strong to show visual associations between peak-force and genotype. The most important genetic effect considered in the literature for

CAST, breed or taurine versus zebu, has been carefully matched so that there are animals of high and of low peak-force from each breed in the sample, and breed is not expected to be an explanatory variable here.

The analysis (Table 3) of the CAST genotypes shows strong, confirmatory evidence of effects of the CAST gene or sequences near the CAST gene on peak-force. The analysis shows no effect of breed, but since the sample consists of individuals of high and low peak-force for each breed, this was not unexpected. The size of effect associated with this polymorphism is approximately 7.9 percent of the phenotypic variance estimated as a main effect, and the deviance associated with CAST genotype nested within breed within region is 121.4 (17 df, P = 0.001894). An un-nested interaction term between breed and CAST genotype was calculated for this sample, but is was not statistically significant. The GLM of the CAST genotypes (Table 4) against the residual peak force measurements show a statistically significant level of association similar to that of the CAST genotypes considered as a main effect (Table 3) rather than when they are nested within region and breed.

A boxplot of genotypes versus the residual peak force measurements (Figure 4) shows clear differences in distributions and the difference between medians of the '11' and the '22' genotypes are approximately 1.2 kg of adjusted peak force. The adjusted means from the analysis of variance (Table 5) gives a difference of 1.34 kg of peak force between the homozygote genotypes. The overall standard deviation for the residuals is 1.61.

The GLM of the confirmatory sample of 77 animals showed a statistically significant association between CAST genotype and peak force, with the '1' allele associated with higher peak forces. When the full model was calculated, none of the factors were statistically significant, possibly as a result of the relatively small sample size. Terms in the model were dropped one by one

using the reduction in AIC as the criterion. All terms except the calpastatin genotypes were dropped (Table 8) in this automatic procedure, and these show a deviance of 17.9 (2 df, $P < 0.05$) explaining 9.5 percent of the phenotypic variance. This is similar to the 8.9 percent found when the CAST genotypes were compared without other factors to peak force in the first sample.

Discussion

The results presented here indicate that genetic variation at the CAST gene is important in explaining variation for Warner-Bratzler peak-force measurements between individuals irrespective of the breed of origin. The sample was chosen to control for the effects of breed and to spread the sample as widely as possible over different sire lines, contemporary groups, feeding and finishing regimes; care was taken to ensure that, as much as possible, individuals in either extreme were from each breed, contemporary group, feeding and finishing system. In this way, systematic effects of these factors on peak-force were controlled so that the effect of the alleles would not be due to inadvertently being carried along by other factors affecting peak-force values. Indeed, there are statistically significant deviations in peak-force due to allelic substitution at this locus even when there is no accounting for the other fixed effects. Inspection of the raw data show frequency differences within breeds for the different genotypes so that the '1' allele is rarer in the extreme with lower peak-force values.

This association between the '1' allele and higher peak force measurements is confirmed in a second smaller sample of extreme animals. These animals are less extreme than those in then first sample, they are the left-over extremes, and they clearly show not only that the calpastatin genotypes are important but that in such a small sample, other factors known to be important are not found to be statistically significant. In a well matched

sample such as this it is not of concern, since we attempt to remove the effects of the other factors as much as possible through the choice of samples to analyse.

The size of the homozygote substitution is approximately 1.34 kg of peak force for the LD, equivalent to 0.83 of standard deviation. This value is likely to be overestimated since the extremes of the distribution were used, and a proper estimate will require animals chosen at random from the full distribution of peak force. Nevertheless, this is a useful amount of genetic variance associated with a single marker and it is expected that this marker would be useful in direct DNA marker tests for breeding and feedlot streaming.

The analysis shows no evidence of a breed by genotype interaction on peak-force, which means that there is no evidence that the allele association is different or absent in some breeds. This is interpreted to mean that there is no heterogeneity in the breeds for the association between calpastatin and peak-force.

A positive test for allelic association generally means that the causative mutation is close to the DNA markers. Associations in other studies have indicated that allelic association decays at an extremely rapid rate so that DNA markers even relatively close to a quantitative trait locus will find no evidence of association (e.g., Coleman et al., 1995; Barendse, 1997). This indicates that the causative mutation or mutations are extremely close to the CAST D/E polymorphism.

Example 3 - Identification of CAST5 microstatellite polymorphism

To determine whether other polymorphisms in the CAST gene are associated with tenderness, both of the cattle samples (Tables 1 & 6) were genotyped with the CAST5 microsatellite polymorphism (Nonneman et al, 1999) which was developed from DNA sequence reported earlier (Cong et al., 1998).

The primer sequences to amplify this polymorphism are

CAST5U1: 5'-GTAAAGCCGCACAAAACACACCCAGG-3' and

CAST5D1: 5'-GTTTCTGGACCCTCTGGATGAGGAAGCGG-3'

with amplification fragments in the range 130 - 159 bp, sizes determined on an ABI 373 DNA sequencer. Alleles and genotypes were assigned based on these size fragments leading to 13 alleles and the distribution of allele sizes is shown in Figure 5.

Two different sets of analyses were performed. In the first, the genotypes at the CAST3 D/E polymorphism were compared to the CAST5 microsatellite to determine whether there were significant associations between the genotypes, as a consequence of haplotypes existing along the DNA sequence. If CAST5 and CAST3 show significant haplotypes, since they are on either side of the CAST coding sequence, then all polymorphisms for the CAST coding sequence are expected to be in linkage disequillibrium with either or both of these DNA markers. In the second, the CAST5 microsatellite alleles were compared to the LD peak-force measurements to determine whether there was any association between CAST5 and tenderness.

Haplotypes between CAST5 and CAST3

Since genotypes of parents of these animals were not available haplotypes where determined by analysing animals in which one or both of CAST5 and CAST3 had homozygous genotypes. The frequency of these haplotypes were tabulated (Table 9). These frequencies were tested for heterogenity using a generalised linear model and found to be highly heterogenous (Table 10). This means that each allele at CAST3 D/E is preferentially associated with specific alleles at the CAST5 microsatellite.

Association between tenderness and CAST5

Since CAST5 has 13 alleles and hence there are 91

possible genotypes, not all of these genotypes will be seen in a sample of 240 samples, as in this study, so the association was estimated on the alleles. As for the CAST3 D/E DNA marker, the polymorphism was compared to the raw LD peak-force values (Table 11 a), was examined for differences in interactions between breeds (Table 11 b), and was compared to the LD peak-force values after market, cohort, breed and finish effects were accounted for (Table 11 c). In the last of these analyses, CAST5 alleles are nested within finish and breed, as in the analysis of CAST3.

These analyses show that there is no interaction between CAST5 allele frequency and breed on LD peak-force, that the association between CAST5 and the raw LD peak-force values is statistically significant at the threshold $P < 0.01$, but when the CAST5 alleles are nested within breed and finish, the association has a deviation which is $0.1 > P > 0.05$. The lack of interaction between CAST5 and breed in explaining LD peak-force means that any differences in gene frequencies between breeds are not responsible for the association between CAST5 and LD peak-force. The association between CAST5 and LD peak-force in sections a and b of Table 11 indicate that there is some evidence for CAST5 associated with LD peak-force, but a bias might still exist, which is why the factors such as market, cohort, breed and finish are also corrected for. Once those factors are corrected, there is a lack of strength in the association. In the CAST3 D/E analysis, correcting the additional factors improved the evidence for the association, and since the same samples are used, we know in which direction the deviations should go. Thus the lack of strength probably means that the large number of alleles nested within breed and finish, has failed to find an association due to the creation of a large number of categories. Larger numbers of alleles are expected to reduce the strength of associations purely due to the number of categories (cf Terwilliger, 1995).

The CAST5 polymorphism can be used in conjunction with the CAST3 D/E polymorphism to predict LD peak-force. For CAST3 D/E the c11 genotype is associated with higher peak force values, the c12 genotype is intermediate and the c22 genotype has the lower peak force values. Secondly, there is linkage disequillibrium between CAST3 D/E and CAST5. By examining the table of haplotypes, looking at the common microsatellite alleles, CAST3 D/E a1 (allele 1) is most often associated with CAST5 m3 (allele 3) with low abundances for m7 and m9. On the contrary, CAST3 D/E a2 (allele 2) is most often associated with CAST5 m9, with a similar large association to m3 and a lesser but still significant association with m7. Inspection of Figure 6, a plot of raw LD peak-force values for each CAST5 microsatellite allele, indicates that CAST5 m7 and m9 have lower peak force values while CAST5 m3 has higher peak force values. Since most of the m3 alleles are actually associated with CAST3 D/E a2 and not a1 (108 versus 14), this higher value is not likely to be the effect of CAST D/E a1. Rather it provides a tool to refine the assignment of animals to groups, since animals selected for having CAST3 D/E a2, so that they might have lower peak force values, might still have higher peak force values if they possessed CAST5 m3. They are expected to have a greater likelihood of having lower peak force values if they possessed both CAST3 D/E a2 as well as CAST5 m7 or m9.

Example 4

This example shows the testing of a DNA marker in the LOX gene for population associations to STIC and STADH. Repeated statistically significant positive associations were found between genotypes and both STIC and STADH. These indicate that, unusually, the heterozygote may be one of the extreme genotypes suggesting some overdominance. These associations are found in a study of 6 breeds of cattle with a structure to detect linkage

disequilibrium and would indicate that the gene LOX either contained or was located near to the genetic factor associated with connective tissue strength.

Materials and Methods

Cattle were chosen from the CRC DNA Bank to have as diverse a genetic and phenotypic background as possible. Two groups of animals were chosen, the first and larger set to test for associations and the second smaller set to confirm the polarity of the associations (cf. Barendse 1997; Barendse et al., 2000). Information stored in the CRC Database was used to select animals. Animals of extremes of instron compression in the semitendinosis muscle were selected. Adhesion measures for these animals were also extracted from the database. In essence, the procedure was to select cattle in each cohort which were of phenotypic extreme measures, to ensure that no sire was represented by a cluster of offspring, that all markets and finishing regimes were included in each extreme, so that extremes were not biased by being representative of a particular market or finishing regime. A total of 253 individuals were selected comprising a first sample of 166 animals and a second sample of 87 animals (Table 11).

The DNA was genotyped for the LOX (Lysyl Oxidase) DNA fragment using the primers LOX K5: 5′ tat cac tga tgt caa acc tg 3′ and LOX K6: 5′ act cag gca cca aat agc tg 3′. The conditions of the polymerase chain reaction (PCR) are an annealing temperature of 60 Celsius, 2.5 mM Magnesium chloride, and reagent mixes obtained from Biotech International. The DNA fragments were labelled via the incorporation of ^{32}P dCTP into the fragments during the PCR. The fragments were digested with HinfI overnight at 37 Celsius before separation on gels. The fragments were visualised via autoradiography to X-Ray film overnight at room temperature. Alleles were scored in

numerical order where the fastest migrating allele is number 1.

The genotypes were analysed using generalised linear models (GLM) following the equation STIC = 1 + genotypes nested within fixed effects + error implemented via the S-PLUS software. The same model is used for STADH. Fixed effects that were considered were breed, finish (Domestic, Korea, Japan), cohort, region (pasture v grain, north v south) and the covariate of age. Age was included since LOX affects cross-linking of collagen and cross-linking is an age related process, with cross-linking increasing over time. The genotypes were nested within region and breed since pure-bred offspring of taurine sires were not pastured in the north. The size of the effects associated with genotype was estimated by the comparison of variances (eg, Andersson-Eklund and Rendel, 1993). To estimate the size of effect associated with genotypic substitution, the same model was fitted without the LOX genotypes. Residuals were extracted and compared to the LOX genotypes. These were analysed using an analysis of variance to obtain adjusted means for each genotype.

Results

There are two alleles (Figure 7) for the LOX polymorphism and both these alleles are found in all the breeds, although there are clear differences in genotype frequency within the breeds. There is no consistent difference between zebu and taurine breeds in frequency of the genotypes (Table 12). The Hereford breed differs radically in genotype frequencies to all the other breeds in the sample. It has high frequencies of genotype '22' while all other breeds have high frequencies of genotype '11'.

The STIC and STADH values are correlated with R=0.52 (Figures 8 - 10). The plots indicate that while the STIC values show two clear extremes the STADH values

have only a long tail and do not show two discrete distributions. This reflects that the sample was selected only on STIC.

The analyses (Tables 13 and 14) of LOX against STIC and STADH show consistent statistically significant associations. The first and the second samples as well as the combined samples of both STIC and STADH show associations to LOX genotypes at $P<0.05$. The association of LOX appears stronger to STADH than to STIC. The association in the second sample of STADH phenotypes has extremely high statistical significance but this may be due to sampling in small populations and the congruence of extreme phenotypes with particular genotypes. The combined analysis of STADH is less extreme than the second sample but shows confirmatory linkage to LOX ($P<0.01$).

Nevertheless, it is clear that these are not large genotypic substitution effects and some analyses do not show statistical significance. When the LOX genotypes were compared to residual STIC and STADH, none of these associations was statistically significant, whether by sample or data combined (Table 15). Interestingly, some of the comparisons show that the heterozygotes are of extreme phenotype, opening the possibility of overdominance at this locus. This will need to be confirmed using other polymorphisms at the LOX gene that show larger genotypic substitution effects.

Discussion

Consistent with those earlier analyses, the STADH values show greater association to the DNA marker than the STIC values, even though the samples are extreme for STIC, with STADH values only more dispersed than normal due to the correlation between traits (Figure 9). STIC was chosen upon which to select extremes rather than STADH. However, both of these measurements evaluate aspects of connective tissue strength, the adhesion measures the force required, in crude terms, to pull a muscle apart,

the force applied perpendicular to the fibre bundles, while the instron compression measures how much the muscle can be flattened without being torn or cut. These are not perfectly correlated as can be seen by inspection of the distribution of STIC and STADH values (Figure 10).

Example 5

The association between the marker and STIC was examined in Example 4 using two batches of extreme animals. The results show that there are significant associations between the genotypes of the marker and STIC (instrom compression, $P < 0.05$), and STADH (adhesion, $P < 0.01$). The results suggest that the gene LOX either contains or is located near the genetic factor associated with connective tissue strength.

Because this study was carried out on a relative small population (253) with extreme animals only, the same marker was tested on different populations to see if the association is still valid.

Materials and Methods

In addition to the population, there are two other groups containing animals chosen from the two tails of instron compression (LDIC, 136) and peak force (LDPF, 131) for the LOX gene study. These three extreme groups together with 559 non-extreme individuals form the base for these analyses on the LOX marker. A total of 917 individuals were used for the study (Table 16).

Due to the nature of the populations, the analyses were carried out to the three datasets.

Extreme animals only (389). The extreme animals from LDIC, LDPF and STPF were pooled together.

Non-extreme animals (559).

Combined data (917). The combination of 1 and 2.

Beside the traits STIC, LDPF and LDIC, a range of other traits was also evaluated to see if there is any effect of LOX gene on other meat quality traits (Table

17). The intramuscular fat measurements from LD_FAT% and NIR_FAT% were combined to make a single trait.

The mixed model procedure (MLX) in SAS (version 8.0) was used to run the statistical analyses. The fixed effects in the model include finish group and LOX marker. Sire and contemporary groups are treated as random effects. All these effects were nested within individual breeds. The statistical model used is as follow:

Trait = mean + sire within breed + contemporary group within breed + finish within breed +LOX within breed +Carcass weight.

Contemporary group was defined as the combination of herd of origin, cohort and kill code. The individuals without electrical stimulation were removed from the analysis data. Carcass weight is being used as a covariate to adjust for the age difference.

A full contrast model would be performed if a significant marker-trait association was identified from a mixed model (or GLM) analysis. The purpose of conducting such the test is to further examine the possibility of additive or dominance or overdominance effect among the genotypes of the LOX marker. The full contrast of 3 genotypes (11, 12 and 22) is set up in SAS as follow:

```
contrast 'Additive Test' lox(bcode) 1 0 -1;
Contrast 'Homozygote1 vs Heterozygote' 1 -1 0
Contrast 'Heterozygote vs Homozygote2' 0 1 -1
contrast 'Dominance Test' lox(bcode) -1 2 -1;
contrast 'Recessive Test' lox(bcode) -1 -1 2;
contrast 'OverDominance Test' lox(bcode) 2 -1 -1;
```

For the extreme population in which the animals with extreme phenotypes were genotyped, multi-trait logistic regression method (Henshall and Goddard, 1999) was applied to take the potential correlation of traits into account. The method is regression based, but instead of regressing phenotype on genotype, the regression is genotype on phenotype. This replaces the assumption that phenotypes are unselected with the assumption that there

was no selection based on genotypes (Henshall and Goddard, 1998). Prior to using logistic regression method, MLX model was used to all data (917 animals) to derive predicted values of individual animals. The predicted phenotype values for the extreme animals after adjusting for significant fixed effects were then used for Logistic regression analyses. The analyses started with single trait logistic regression method and then proceeded to multi-trait logistic regression method.

The genotype frequency distribution of the marker in different populations is shown in Table 18. From the table, it can be seen that the Hereford breed differs remarkably in genotype frequencies to all the other breeds in the populations. In order to investigate the potential effect of skewed genotypes of Hereford breed on the overall results, a set of additional analyses were also pursued to the populations by removing the Hereford individuals from the data sets.

Results and Discussions

Part I. Extreme Animals (Table 19)

Extreme animals for LDIC

The first test was conducted to the sample containing the selected animals for LDIC (136). The results from the analysis of variance reveal that there was no close association between any genotypes of LOX marker and LDIC. The same conclusion was held to other meat quality traits.

Extreme animals for LDPF

Like LDIC sample, there was no significant variation detected between the LOX marker and any meat quality trait in the batch animals selected for LDPF. The results are not surprising as the initial QTL for tenderness in CBX experiment was identified in instron compression measurement of Semitendinosus muscles.

Combined extreme animal data

Analysis of Variance. As sire effect was confounded with other effects, it had to be removed from the model and GLM (generalised linear model) was performed. In this case, contemporary group was treated as a fixed effect rather than a random effect. The analysis of variance has shown that out of 21 meat quality traits tested, STIC and LDL had significant results ($P < 0.05$).

Full Contrast Model. The results from full contrast model are given below. For STIC, it can be seen that there was no additive effect between the two homozygous genotypes (11 and 22). However, the highly significant difference between the phenotypes of 11 and 12 obviously contributed to the detection of dominant and overdominant effects. Nothing was remarkable for LDL.

Logistic Regression. After adjusting for the significant fixed effects on all data, logistic regression was applied to STIC. Multi-trait logistic regression model was also fitted to take the potential correlations between ST measurements into account (STIC, STPF and STADH). The results confirm the findings from the other methods. That is, LOX genotypes did have a correlation with STIC. The regression co-efficiency between lox marker and STIC is shown in the output of logistic procedure (below). The allele substitution effect of the lox marker could be derived from the formulae suggested by Henshall and Goddard (1999) based on the total variance of whole data. The multi-trait logistic regression test on STIC, STPF and STADH has shown that both STIC and STPF had significant effects on LOX gene marker. STPF was marginally non-significant in GLM analysis. (Table 20)

Part II. CRC Non-Extreme Animals

The non-extreme animals (559) were genotyped against LOX marker in CRC I and but were not part of the animals involved in marker evaluation Phases III. The mixed model analyses of variance show that beside STIC,

the significant marker-trait association was also detected to the intramuscular fat (FAT) and LDPH. However, full contrast test to STIC and FAT has failed to pinpoint the genotype causing the significant results. In the case of LDPH, it seems that 22 genotype had an important role in determining the outcomes. (Table 21)

Part III. Combined Data

When extreme and non-extreme animals were pooled together, the results from mixed model analysis of variance show that again the lox marker was associated with STIC ($P < 0.05$). The significant results were also found in STL, which is the measurement of darkness of carcass meat colour. However in both cases, full contrast model had not be able to identify the significant genotype sources. (Table 22)

Part IV. Removing Hereford individuals from the combined population

In order to test the possible effect of skewed distribution of lox genotypes of Hereford breed, the additional analyses were also performed to the combined data with the removal of Hereford breed. The results indicate that the removal of Hereford animals has changed little to the overall significant results of STIC in the combined population. From the genotype frequency distribution table, it can be seen that the majority of Hereford individuals were from the three extreme populations except one animal from non-extreme CRC population. (Table 23)

The overall results from the investigation of LOX gene effect on meat quality traits have been consistent across three populations (extreme, non-extreme and combined). That is, there is a strong association of LOX gene marker with the instron compression measurement of Semitendinosus muscles ($P < 0.05$). The significant

results from other meat quality traits vary from one population to another.

Industrial Applicability

The invention is useful in allowing selection and breeding of animals which yield more tender meat.

Table 1

Characteristics of the first Cattle Sample

Total: 169		
	83	high peak force
	86	low peak force
Breeds:	29	Santa Gertrudis
	25	Hereford
	26	Angus
	27	Belmont Red
	31	Brahman
	31	Shorthorn
Regions:	38	Pasture South
	28	Pasture North
	57	Grain South
	41	Grain North
Markets:	72	Korean
	67	Domestic
	25	Japanese
Cohorts:	27	Cohorts
	Median: 5 steers per cohort	
	bottom quartile: 2 steers per cohort	
	top quartile: 9 steers per cohort	
Sires:	112	sires
	Median: 1 steer per sire	
	bottom quartile: 1 steer per sire	
	top quartile: 2 steers per sire	

Table 2

Distribution of CAST genotypes in the breeds in the first sample.

Breed	Genotype			
	11	12	22	23
Angus	0	7	19	0
Belmont Red	0	8	19	0
Brahman	6	13	10	2
Hereford	0	5	17	0
Santa Gertrudis	3	5	19	0
Shorthorn	0	4	23	0

Table 3

Associations between calpastatin genotypes (castg) and tenderness.

A. Calpastatin by itself

Analysis of Deviance Table

Gaussian model

Response: peakforce

Terms added sequentially (first to last)

	Df	Deviance	Resid. Df	Resid. Dev	F Value	Pr(F)
NULL			155	864.6307		
castg	3	70.89899	152	793.7317	4.52573	0.004536025

B. Breed x Calpastatin Interactions

Analysis of Deviance Table

Gaussian model

Response: peakforce

Terms added sequentially (first to last)

	Df	Deviance	Resid. Df	Resid. Dev	F Value	Pr(F)
NULL			155	864.6307		
finish	2	101.8455	153	762.7852	15.97637	0.0000008
cohort	25	273.5882	128	489.1971	3.43339	0.0000041
region	3	59.6620	125	429.5350	6.23940	0.0005889
breed	4	10.8711	121	418.6639	0.85267	0.4948672
castg	3	28.2709	118	390.3930	2.95654	0.0355308
breed:castg	6	33.4066	112	356.9864	1.74682	0.1166518

Table 3 (continued)

C. Calpastatin genotypes nested with breed and region

Analysis of Deviance Table

Gaussian model

Response: peakforce

Terms added sequentially (first to last)

	Df	Deviance	Resid. Df	Resid. Dev	F Value	Pr(F)
NULL			155	864.6307		
finish	2	101.8455	153	762.7852	18.28842	0.0000002
cohort	25	273.5882	128	489.1971	3.93027	0.0000005
region	3	59.6620	125	429.5350	7.14235	0.0002128
breed in region	7	26.8943	118	402.6408	1.37983	0.2219802
castg in (region/breed)	17	121.4139	101	281.2269	2.56498	0.0018938

Table 4

Analysis of CAST against residual peakforce measurements (X1).

Analysis of Deviance Table

Gaussian model

Response: X1

Terms added sequentially (first to last)

	Df	Deviance	Resid. Df	Resid. Dev	F Value	Pr(F)
NULL			154	402.3160		
castg	3	21.97947	151	380.3365	2.90874	0.03654659

Call:

glm(formula = X1 castg, data = calppftest, na.action = na.omit)

Coefficients:

(Intercept)	castg1	castg2	castg3
0.07693593	-0.4095948	-0.3102917	-0.3504961

Degrees of Freedom: 155 Total; 151 Residual

Residual Deviance: 380.3365

Model from which residuals were calculated

Analysis of Deviance Table

Gaussian model

Response: peakforce

Terms added sequentially (first to last)

	Df	Deviance	Resid. Df	Resid. Dev	F Value	Pr(F)
NULL			160	906.5175		
finish	2	106.0217	158	800.4958	15.52293	0.0000010
cohort	26	290.8387	132	509.6571	3.27558	0.0000057
finlwt	1	8.0662	131	501.5908	2.36200	0.1269337
region	3	63.9431	128	437.6478	6.24139	0.0005629
breed in region	7	24.4323	121	413.2154	1.02206	0.4192678

glm(formula: peakforce = finish + cohort + finlwt + region/breed,

data = calppf, na.action = na.omit)

Table 5

Analysis of Variance tables between CAST genotypes and residual peak force measures (X1) along with the table of adjusted means associated with each genotype.

Analysis of Variance Table

Response: X1

Terms added sequentially (first to last)

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
castg	3	21.9795	7.326490	2.90874	0.03654659
Residuals	151	380.3365	2.518785		

Tables of adjusted means

Grand mean
0.076936
se 0.318703

castg

	c11	c12	c22	c23
	1.1473	0.3281	-0.1932	-0.9746
se	0.5290	0.2479	0.1564	1.1222

Table 6

Characteristics of the second sample of 77 animals.

Total:	77	
	39	high peak force
	38	low peak force
Breeds:	11	Belmont Red
	11	Hereford
	13	Brahman
	13	Shorthorn
	14	Santa Gertrudis
	15	Angus
Regions:	24	Pasture South
	12	Pasture North
	21	Grain South
	20	Grain North
Markets:	35	Korean
	25	Domestic
	17	Japanese
Cohorts:	22	Cohorts
	Median: 3 steers per cohort	
	bottom quartile: 2 steers per cohort	
	top quartile: 5 steers per cohort	
Sires:	64	sires
	Median: 1 animal per sire	
	bottom quartile: 1 animal per sire	
	top quartile: 1 animal per sire	

Table 7

Distribution of CAST genotypes in the second sample

Breed	Genotype		
	11	12	22
Angus	0	3	12
Belmont Red	0	3	8
Brahman	3	7	3
Hereford	0	3	8
Santa Gertrudis	1	4	9
Shorthorn	0	0	13

Table 8

Associations between calpastatin genotypes and LD peak force in the second sample.

Analysis of Deviance Table

Gaussian model

Response: ldpeakforce

Terms added sequentially (first to last)

	Df	Deviance	Resid. Df	Resid. Dev	F Value	Pr(F)
NULL			76	205.9332		
castg	2	17.90313	74	188.0300	3.522925	0.03455689

Coefficients:

(Intercept)	castg1	castg2
5.227591	-0.1205	-0.3719088

Degrees of Freedom: 77 Total; 74 Residual

Residual Deviance: 188.03

Single term deletions

Model:
ldpeakforce = lslortwait + buttemp + finish + cohort + region + breed + castg

Final Call:

glm(formula = ldpeakforce castg, data = calppfr, na.action = na.omit)

Coefficients:

(Intercept)	castg1	castg2
5.195064	-0.07055556	-0.37438

Degrees of Freedom: 67 Total; 64 Residual

Residual Deviance: 161.5878

Table 9

The amount of each haplotype found between the alleles of the cast5 microsatellite and the cast3 D/E SNP on both cattle samples.

Twenty-six haplotypes were found in animals that are homozygous for one or the other locus.

haplotype	allele cast3	cast5	amount
1	a1	m1	3
2	a1	m2	0
3	a1	m3	14
4	a1	m4	0
5	a1	m5	0
6	a1	m6	3
7	a1	m7	6
8	a1	m8	2
9	a1	m9	6
10	a1	m10	5
11	a1	m11	0
12	a1	m12	1
13	a1	m13	0
14	a2	m1	0
15	a2	m2	1
16	a2	m3	108
17	a2	m4	1
18	a2	m5	4
19	a2	m6	3
20	a2	m7	42
21	a2	m8	2
22	a2	m9	110
23	a2	m10	17
24	a2	m11	6
25	a2	m12	1
26	a2	m13	1

Table 10

A heterogeneity test for associations between alleles at CAST5 with alleles at CAST3 D/E.

Analysis of Deviance Table

Poisson model

Response: score

Terms added sequentially (first to last)

	Df	Deviance	Resid. Df	Resid. Dev	Pr(Chi)
NULL			25	926.9836	
cast3	1	220.4994	24	706.4842	0.0000000000
cast5	12	671.7497	12	34.7345	0.0000000000
cast3:cast5	12	34.7344	0	0.0001	0.0005161421

Table 11

Tests for association between CAST 5 microsatellite and LD peak force measurements in both cattle samples.

Part A. Calpastatin by itself

Analysis of Deviance Table

Gaussian model

Response: ldpf

Terms added sequentially (first to last)

	Df	Deviance	Resid. Df	Resid. Dev	F Value	Pr(F)
NULL			491	2266.640		
mall1	12	136.0104	479	2130.629	2.548113	0.002843108

Part B. Breed by calpastatin interactions

Analysis of Deviance Table

Table 10 (Continued)

Gaussian model

Response: ld

Terms added sequentially (first to last)

	Df	Deviance	Resid. Df	Resid. Dev	F Value	Pr(F)
NULL			491	2266.640		
market	2	218.4756	489	2048.164	42.35776	0.0000000
cohort	26	706.3980	463	1341.766	10.53504	0.0000000
finish	3	97.7159	460	1244.050	12.63003	0.0000001
breed	4	23.5779	456	1220.472	2.28562	0.0594827
cast5	12	65.7617	444	1154.711	2.12497	0.0146070
breed:cast5	26	76.7170	418	1077.994	1.14414	0.2865614

Part C. Calpastatin genotypes nested with breed and finish (region)

Analysis of Deviance Table

Gaussian model

Response: ld

Terms added sequentially (first to last)

	Df	Deviance	Resid. Df	Resid. Dev	F Value	Pr(F)
NULL			491	2266.640		
market	2	218.4756	489	2048.164	42.90121	0.00000000
cohort	26	706.3980	463	1341.766	10.67020	0.00000000
breed	4	30.7280	459	1311.038	3.01697	0.01799825
finish %in% reed	6	111.2067	453	1199.832	7.27908	0.00000022
cast5 %in% (breed/finish)	65	211.8811	388	987.950	1.28019	0.08307834

Table 11

Characteristics of the Cattle Sample

Total:	166	87
high instron compression	87	39
low instron compression	89	38
Breeds:		
Angus	25	12
Belmont Red	25	12
Brahman	33	18
Hereford	32	10
Santa Gertrudis	26	15
Shorthorn	25	17
Regions:		
Pasture South	47	20
Pasture North	39	21
Grain South	43	20
Grain North	37	26
Markets:		
Korean	81	22
Domestic	47	45
Japanese	38	22
Cohorts:	25	14
Median: steers per cohort	6	3
bottom quartile:	3	1
top quartile:	10	11
Sires:	113	62
Median: steers per sire	1	1
bottom quartile:	1	1
top quartile:	2	2

Table 12

Distribution of LOX genotypes in the breeds in the combined sample.

Breed	Genotype		
	11	12	22
Angus	12	16	5
Belmont Red	19	14	2
Brahman	20	21	4
Hereford	1	7	27
Santa Gertrudis	18	5	1
Shorthorn	23	11	3

Table 13

Associations between LOX genotypes (loxg) and STIC.

A. First Sample

Analysis of Deviance Table

Gaussian model

Response: stic

Terms added sequentially (first to last)

	Df	Deviance	Resid.Df	Resid. Dev	F Value	Pr(F)
NULL			144	66.41782		
market	2	4.68851	142	61.72932	10.11346	0.0001124
age	1	0.33061	141	61.39871	1.42631	0.2356143
cohort	23	25.50396	118	35.89474	4.78382	0.0000000
region	3	2.05111	115	33.84363	2.94960	0.0371506
breed %in% region	8	3.73686	107	30.10677	2.01517	0.0537829
loxg %in% (region/breed)	20	9.94057	87	20.16620	2.14425	0.0081786

B. Second Sample

Analysis of Deviance Table

Gaussian model

Response: stic

Terms added sequentially (first to last)

	Df	Deviance	Resid.Df	Resid. Dev	F Value	Pr(F)
NULL			77	36.73118		
market	2	18.56015	75	18.17103	50.57448	0.0000000
age	1	1.22303	74	16.94800	6.66525	0.0131586
region	3	0.49228	71	16.45571	0.89428	0.4514796
breed %in% region	9	2.26136	62	14.19435	1.36933	0.2303598
loxg %in% (region/breed)	17	5.93715	45	8.25720	1.90331	0.0433708

NOTE: cohort could not be fitted as it required a model with more terms than degrees of freedom. cohort was dropped since that allowed a maximum of other terms to be fitted.

C. Combined Sample

Analysis of Deviance Table

Gaussian model

Response: stic

Terms added sequentially (first to last)

	Df	Deviance	Resid.Df	Resid. Dev	F Value	Pr(F)
NULL			222	105.0131		
market	2	9.28932	220	95.7238	14.63402	0.0000015
age	1	0.66404	219	95.0598	2.09221	0.1500079
cohort	23	28.46167	196	66.5981	3.89890	0.0000002
region	3	0.97412	193	65.6240	1.02306	0.3840448
breed %in% region	9	2.08684	184	63.5372	0.73056	0.6804229
loxg %in% (region/breed)	24	12.75507	160	50.7821	1.67448	0.0327546

Table 14

Associations between LOX genotypes (loxg) and STADH.

A. First Sample

Analysis of Deviance Table

Gaussian model

Response: stadh

Terms added sequentially (first to last)

	Df	Deviance	Resid.Df	Resid. Dev	F Value	Pr(F)
NULL			139	4.118160		
market	2	0.045611	137	4.072549	1.63665	0.2008490
age	1	1.030876	136	3.041674	73.98193	0.0000000
cohort	23	1.131428	113	1.910246	3.53035	0.0000129
region	3	0.053546	110	1.856699	1.28094	0.2863452
breed %in% region	8	0.192061	102	1.664639	1.72293	0.1050769
loxg %in% (region/breed)	19	0.508104	83	1.156535	1.91919	0.0229812

B. Second Sample

Analysis of Deviance Table

Gaussian model

Response: stadh

Terms added sequentially (first to last)

	Df	Deviance	Resid.Df	Resid. Dev	F Value	Pr(F)
NULL			76	3.362345		
market	2	0.560443	74	2.801903	25.18352	0.0000000513
age	1	0.486387	73	2.315516	43.71164	0.0000000425
region	3	0.390962	70	1.924554	11.71193	0.0000090738
breed %in% region	9	0.413632	61	1.510922	4.13035	0.0006637134
loxg %in% (region/breed)	17	1.021326	44	0.489595	5.39922	0.0000032684

C. Combined Sample

Analysis of Deviance Table

Gaussian model

Response: stadh

Terms added sequentially (first to last)

	Df	Deviance	Resid.Df	Resid. Dev	F Value	Pr(F)
NULL			216	7.480742		
market	2	0.268508	214	7.212234	7.78709	0.0005990
age	1	1.252352	213	5.959882	72.63985	0.0000000
cohort	23	2.111912	190	3.847971	5.32594	0.0000000
region	3	0.065737	187	3.782233	1.27098	0.2863505
breed %in% region	9	0.325818	178	3.456415	2.09982	0.0326124
loxg %in% (region/breed)	23	0.784128	155	2.672287	1.97746	0.0079540

Table 15

Estimated sizes of effects of genotype substitutions at LOX on instron compression and adhesion of the semitendinosus muscle.

Response: stic.resid				
Grand-mean	0.027133		se	0.053648
Loxg		111	112	122
		0.03958	-0.02321	0.06503
	se	0.07654	0.07897	0.11751
Response: sticbox3.resid				
Grand-mean	-0.012888		se	0.039137
Loxg		111	112	122
		0.033317	-0.058769	-0.013213
	se	0.055916	0.058431	0.085115
Response: sticfull.resid				
Grand-mean	-0.012888		se	0.039137
loxg		111	112	122
		0.033317	-0.058769	-0.013213
	se	0.055916	0.058431	0.085115
Response: sticadh.resid				
Grand-mean	0.002521		se	0.010321
Loxg		111	112	122
		-0.001426	-0.002304	0.011292
	se	0.014860	0.015390	0.022384
Response: sticbox3adh.resid				
Grand-mean	0.004308		se	0.012879
loxg		111	112	122
		-0.003154	-0.007446	0.023524
	se	0.018594	0.018891	0.028111

Response: sticfulladh.resid			
Grand-mean	0.0037399	se	0.0092835
loxg	111	112	122
	-0.009303	-0.005244	0.025767
	se 0.013379	0.013763	0.020180

Table 16

Information on the data sets

Effect	Class	LDIC	LDPF	Combined Extreme	Non-extreme	Combined
Total		136	131	398	543	916
Sires		96	96	171	61	227
Cohorts		24	24	26	10	30
Breeds	Angus	19	22	62	134	196
	Belmont Red	23	25	66	140	200
	Brahman	24	27	76	73	142
	Hereford	27	14	67	1	68
	Santa Gertrudis	25	24	73	195	257
	Shorthorn	18	19	53	0	53
Regions	Pasture South	27	27	92	93	185
	Pasture North	24	25	78	144	212
	Grain South	54	41	125	168	291
	Grain North	31	38	102	138	228
Markets	Domestic	47	51	130	240	361
	Korean	61	58	189	201	376
	Japaness	28	22	78	102	179

Table 17

Meat quality traits tested for LOX gene marker

Code	Trait
LD_Fat%	Intramuscular Fat percentage (Soxhylet Method)
LD_IC	Longissimus dorsi Instrom compression
LD_IY	Longissimus dorsi initial yield (Nth kills only)
LD_LOSS	Longissimus dorsi cooking loss%
LD_PF	Longissimus dorsi Peak Force -must use "Stim" also
LD_PF-IY	Longissimus dorsi Peak Force - initial yield (Nth)
LD_a	Longissimus dorsi a* colour
LD_b	Longissimus dorsi b* colour
LD_l	Longissimus dorsi L* colour
LD_pH	Longissimus dorsi ultimate pH
NIR_Fat%	Intramuscular Fat percentage (NIR method)
ST_AdhRS	Semitendinosus Shorthose adhesion
ST_IC	Semitendinosus Instrom compression
ST_IY	Semitendinosus initial yield (Nth kills only)
ST_LOSS	Semitendinosus cooking loss%
ST_PF	Semitendinosus Peak Force
ST_PF-IY	Semitendinosus Peak Force - initial yield (Nth)
ST_a	Semitendinosus a* colour
ST_b	Semitendinosus b* colour
ST_l	Semitendinosus L* colour
ST_pH	Semitendinosus ultimate pH
TenderQ	Tenderness Quality as measured by PF (x 100)

Table 18

Distribution of LOX genotypes in the breeds in the three datasets

Breed	Extreme			Non-extreme			Combined		
	11	12	22	11	12	22	11	12	22
Angus	21	33	7	38	75	21	59	109	28
Brahman	35	35	6	19	40	14	52	71	19
Belmont Red	31	23	12	59	60	21	87	83	30
Hereford	4	17	46	0	1	0	4	18	46
Santa Gertrudis	42	29	2	120	62	13	156	86	15
Shorthorn	34	18	1	0	0	0	34	18	1
Total	167	156	74	236	238	69	392	385	139

Table 19

The GLM Procedure

Dependent Variable: STIC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	297	90.3108958	0.3040771	2.54	<.0001
Error	97	11.5922526	0.1195078		
Corrected Total	394	101.9031484			

R-Square	Coeff Var	Root MSE	STIC Mean
0.886242	16.41081	0.345699	2.106532

Source	DF	Type III SS	Mean Square	F Value	Pr > F
contemp(Bcode)	269	72.05453149	0.26786071	2.24	<.0001
Fingp(Bcode)	3	0.56463473	0.18821158	1.57	0.2004
Stim	1	0.29426415	0.29426415	2.46	0.1199
lox(Bcode)	12	3.23498136	0.26958178	2.26	0.0145
wt	1	0.62547588	0.62547588	5.23	0.0243

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
Additive Test 11-22	1	0.42074192	0.42074192	3.52	0.0636
11-12	1	2.17031475	2.17031475	18.16	<.0001
12-22	1	0.17567132	0.17567132	1.47	0.2283
Dominance Test	1	0.97536352	0.97536352	8.16	0.0052
Recessive Test	1	0.00964320	0.00964320	0.08	0.7770
OverDominance Test	1	1.58776355	1.58776355	13.29	0.0004

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Additive Test 11-22	-0.54698783	0.29151964	-1.88	0.0636
11-12	-0.93226650	0.21876446	-4.26	<.0001
12-22	0.38527867	0.31777709	1.21	0.2283
Dominance Test	1.31754517	0.46119045	2.86	0.0052
Recessive Test	0.16170916	0.56927504	0.28	0.7770
OverDominance Test	-1.47925433	0.40583359	-3.64	0.0004

LDL

The GLM Procedure

Dependent Variable: LDl

Table 19 (Continued)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	295	4070.356476	13.797819	1.96	<.0001
Error	96	675.059834	7.031873		
Corrected Total	391	4745.416310			

R-Square	Coeff Var	Root MSE	LDl Mean
0.857745	6.920726	2.651768	38.31633

Source	DF	Type III SS	Mean Square	F Value	Pr > F
contemp(Bcode)	267	2628.383772	9.844134	1.40	0.0278
Fingp(Bcode)	3	25.195055	8.398352	1.19	0.3161
Stim	1	0.195851	0.195851	0.03	0.8678
lox(Bcode)	12	160.954945	13.412912	1.91	0.0427
wt	1	5.974613	5.974613	0.85	0.3590

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
Additive Test	1	5.88718974	5.88718974	0.84	0.3625
11 vs 12	1	3.08780445	3.08780445	0.44	0.5091
12 vs 22	1	1.03256209	1.03256209	0.15	0.7024
Dominance Test	1	0.01778689	0.01778689	0.00	0.9600
Recessive Test	1	3.27515251	3.27515251	0.47	0.4966
OverDominance Test	1	7.23682011	7.23682011	1.03	0.3129

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Additive Test 11-22	-2.04608559	2.23617248	-0.91	0.3625
11-12	-1.11200497	1.67809812	-0.66	0.5091
12-22	-0.93408062	2.43759636	-0.38	0.7024
Dominance Test	0.17792435	3.53769859	0.05	0.9600
Recessive Test	2.98016622	4.36676923	0.68	0.4966
OverDominance Test	-3.15809056	3.11305081	-1.01	0.3129

Table 20 (continued)

Single Trait Logistic Regression

The LOGISTIC Procedure

Model Information

Data Set	WORK.EXTRERES
Response Variable	lox
Number of Response Levels	3
Number of Observations	389
Link Function	Logit
Optimization Technique	Fisher's scoring

Response Profile

Ordered Value	lox	Total Frequency
1	11	165
2	12	153
3	22	71

NOTE: 8 observations were deleted due to missing values for the response or explanatory variables.

The LOGISTIC Procedure

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	5.0720	1	0.0243
Score	5.0502	1	0.0246
Wald	5.0083	1	0.0252

Analysis of Maximum Likelihood Estimates

Parameter	DF	Estimate	Standard Error	Chi-Square	Pr > ChiSq
Intercept	1	0.9245	0.5608	2.7174	0.0993
Intercept2	1	2.7482	0.5777	22.6278	<.0001
sticpred	1	-0.5827	0.2604	5.0083	0.0252

Odds Ratio Estimates

Effect	Point Estimate	95% Wald Confidence Limits	
sticpred	0.558	0.335	0.930

Table 20 (continued)

Multi-trait Logistic Regression

The LOGISTIC Procedure

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	14.7234	3	0.0021
Score	14.5859	3	0.0022
Wald	13.9424	3	0.0030

Analysis of Maximum Likelihood Estimates

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept 11	1	-0.9210	0.8702	1.1202	0.2899
Intercept 12	1	0.9467	0.8711	1.1813	0.2771
sticpred	1	-0.8681	0.3191	7.4014	0.0065
stadhpred	1	-0.4025	0.7724	0.2715	0.6023
stpfpred	1	0.5689	0.1936	8.6313	0.0033

Odds Ratio Estimates

Effect	Point Estimate	95% Wald Confidence Limits	
sticpred	0.420	0.225	0.785
stadhpred	0.669	0.147	3.039
stpfpred	1.766	1.208	2.581

Table 21

The Mixed Procedure

Model Information

Data Set	WORK.CRC1
Dependent Variable	STIC
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Covariance Parameter Estimates

Cov Parm	Estimate	Standard Error	Z Value	Pr Z
SireID(Bcode)	0.002544	0.002147	1.18	0.1181
contemp(Bcode)	0.008813	0.003896	2.26	0.0118
Residual	0.07009	0.005174	13.55	<.0001

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Fingp(Bcode)	9	317	17.50	<.0001
Stim	1	317	1.75	0.1870
lox(Bcode)	8	317	2.36	0.0176
wt	1	317	6.45	0.0115

Estimates

Label	Estimate	Standard Error	DF	t Value	Pr > |t|
Additive Test 11-22	0.05547	0.07813	317	0.71	0.4782
11-12	0.1044	0.05660	317	1.84	0.0661
12-22	-0.04890	0.07013	317	-0.70	0.4861
Dominance Test	-0.1533	0.1007	317	-1.52	0.1289
Recessive Test	-0.00657	0.1373	317	-0.05	0.9619
OverDominance Test	0.1598	0.1170	317	1.37	0.1730

Contrasts

Label	Num DF	Den DF	F Value	Pr > F
Additive Test 11-22	1	317	0.50	0.4782
11-12	1	317	3.40	0.0661
12-22	1	317	0.49	0.4861
Dominance Test	1	317	2.32	0.1289
Recessive Test	1	317	0.00	0.9619
OverDominance Test	1	317	1.87	0.1730

Instramuscuar Fat

The Mixed Procedure

Model Information

Data Set	WORK.CRC1
Dependent Variable	Fat
Covariance Structure	Variance Components

Table 21 (continued)

Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Covariance Parameter Estimates

Cov Parm	Estimate	Standard Error	Z Value	Pr Z
SireID(Bcode)	0.04848	0.04510	1.07	0.1412
contemp(Bcode)	0.3111	0.1060	2.93	0.0017
Residual	1.2479	0.09791	12.75	<.0001

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Fingp(Bcode)	9	306	7.08	<.0001
Stim	1	306	0.31	0.5790
lox(Bcode)	8	306	2.00	0.0461
wt	1	306	74.54	<.0001

Estimates

Label	Estimate	Standard Error	DF	t Value	Pr > |t|
Additive Test 11-22	0.3765	0.3427	306	1.10	0.2728
11-12	-0.1207	0.2505	306	-0.48	0.6304
12-22	0.4972	0.3029	306	1.64	0.1018
Dominance Test	0.6179	0.4377	306	1.41	0.1591
Recessive Test	-0.8737	0.5964	306	-1.47	0.1439
OverDominance Test	0.2558	0.5184	306	0.49	0.6220

Contrasts

Label	Num DF	Den DF	F Value	Pr > F
Additive Test 11-22	1	306	1.21	0.2728
11-12	1	306	0.23	0.6304
12-22	1	306	2.69	0.1018
Dominance Test	1	306	1.99	0.1591
Recessive Test	1	306	2.15	0.1439
OverDominance Test	1	306	0.24	0.6220

LDPH

The Mixed Procedure

Model Information

Data Set	WORK.CRC1
Dependent Variable	LDpH
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Covariance Parameter Estimates

	Standard	Z

Table 21 (continued)

Cov Parm	Estimate	Error	Value	Pr Z
contemp(Bcode)	0.002771	0.000702	3.95	<.0001
Residual	0.007878	0.000572	13.77	<.0001

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Fingp(Bcode)	9	363	3.44	0.0004
Stim	1	363	1.14	0.2854
lox(Bcode)	8	363	3.01	0.0027
wt	1	363	21.61	<.0001

Estimates

Label	Estimate	Standard Error	DF	t Value	Pr > |t|
Additive Test 11-22	-0.1089	0.02648	363	-4.11	<.0001
11-12	-0.01502	0.01922	363	-0.78	0.4351
12-22	-0.09393	0.02376	363	-3.95	<.0001
Dominance Test	-0.07891	0.03416	363	-2.31	0.0215
Recessive Test	0.2029	0.04650	363	4.36	<.0001
OverDominance Test	-0.1240	0.03971	363	-3.12	0.0019

The Mixed Procedure

Contrasts

Label	Num DF	Den DF	F Value	Pr > F
Additive Test 11-22	1	363	16.93	<.0001
11-12	1	363	0.61	0.4351
12-22	1	363	15.63	<.0001
Dominance Test	1	363	5.34	0.0215
Recessive Test	1	363	19.04	<.0001
OverDominance Test	1	363	9.75	0.0019

Table 22

STIC

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Estimate	Standard Error	Z Value	Pr Z
SireID(Bcode)	0.006141	0.004815	1.28	0.1011
contemp(Bcode)	0.05433	0.01003	5.42	<.0001
Residual	0.09190	0.006489	14.16	<.0001

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Fingp(Bcode)	11	369	9.24	<.0001
Stim	2	369	4.06	0.0181
lox(Bcode)	12	369	1.90	0.0336
wt	1	369	9.93	0.0018

Estimates

Label	Estimate	Standard Error	DF	t Value	Pr > |t|
Additive Test 11-22	0.06327	0.08142	369	0.78	0.4376
11-12	0.07985	0.05752	369	1.39	0.1659
12-22	-0.01658	0.07440	369	-0.22	0.8238
Dominance Test	-0.09643	0.1052	369	-0.92	0.3598
Recessive Test	-0.04669	0.1450	369	-0.32	0.7476
OverDominance Test	0.1431	0.1198	369	1.20	0.2328

Contrasts

Label	Num DF	Den DF	F Value	Pr > F
Additive Test 11-22	1	369	0.60	0.4376
11-12	1	369	1.93	0.1659
12-22	1	369	0.05	0.8238
Dominance Test	1	369	0.84	0.3598
Recessive Test	1	369	0.10	0.7476
OverDominance Test	1	369	1.43	0.2328

STL

Covariance Parameter Estimates

Cov Parm	Estimate	Standard Error	Z Value	Pr Z
SireID(Bcode)	0.4389	0.3429	1.28	0.1003
contemp(Bcode)	3.3608	0.7375	4.56	<.0001
Residual	10.6090	0.6789	15.63	<.0001

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Fingp(Bcode)	11	368	16.40	<.0001
Stim	2	368	4.68	0.0098
lox(Bcode)	12	368	2.18	0.0124

Table 22 (Continued)

wt	1	368	8.64	0.0035	

Estimates

Label	Estimate	Standard Error	DF	t Value	Pr > |t|
Additive Test 11-22	-0.7500	0.8582	368	-0.87	0.3827
11-12	-0.6732	0.5998	368	-1.12	0.2625
12-22	-0.07683	0.7815	368	-0.10	0.9217
Dominance Test	0.5963	1.0975	368	0.54	0.5872
Recessive Test	0.8268	1.5279	368	0.54	0.5887
OverDominance Test	-1.4232	1.2576	368	-1.13	0.2585

Contrasts

Label	Num DF	Den DF	F Value	Pr > F
Additive Test 11-22	1	368	0.76	0.3827
11-12	1	368	1.26	0.2625
12-22	1	368	0.01	0.9217
Dominance Test	1	368	0.30	0.5872
Recessive Test	1	368	0.29	0.5887
OverDominance Test	1	368	1.28	0.2585

Table 23

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Estimate	Standard Error	Z Value	Pr Z
SireID(Bcode)	0.005452	0.004008	1.36	0.0869
contemp(Bcode)	0.03915	0.008774	4.46	<.0001
Residual	0.09105	0.006426	14.17	<.0001

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Fingp(Bcode)	10	368	10.46	<.0001
Stim	2	368	5.89	0.0030
lox(Bcode)	10	368	2.28	0.0132
wt	1	368	7.14	0.0079

Estimates

Label	Estimate	Standard Error	DF	t Value	Pr > |t|
Additive Test 11-22	0.06263	0.07965	368	0.79	0.4322
11-12	0.09181	0.05618	368	1.63	0.1031
12-22	-0.02918	0.07277	368	-0.40	0.6887
Dominance Test	-0.1210	0.1028	368	-1.18	0.2398
Recessive Test	-0.03345	0.1419	368	-0.24	0.8137
OverDominance Test	0.1544	0.1171	368	1.32	0.1879

Contrasts

Label	Num DF	Den DF	F Value	Pr > F
Additive Test 11-22	1	368	0.62	0.4322
11-12	1	368	2.67	0.1031
12-22	1	368	0.16	0.6887
Dominance Test	1	368	1.39	0.2398
Recessive Test	1	368	0.06	0.8137
OverDominance Test	1	368	1.74	0.1879

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Claims:

1. A method for assessing the tenderness of meat from an animal, comprising the step of testing the animal for the presence or absence of a genetic marker selected from the group consisting of:

(1) an allele of the gene encoding calpastatin (CAST) associated with peak-force variation or genetic variation located other than in the CAST gene which shows allelic association with the CAST allele; and

(2) an allele of the gene encoding lysyl oxidase (LOX) associated with variation in instron compression of the semitendinosis muscle or genetic variation located other than in the LOX gene which shows allelic association with the LOX allele.

2. A method as claimed in claim 1 wherein the genetic marker is located in the 3′ UTR of the CAST gene.

3. A method as claimed in claim 2 wherein the allele is CAST3 D/E allele 1.

4. A method as claimed in claim 2 wherein the allele is CAST3 D/E allele 2 or allele 3.

5. A method as claimed in claim 1 wherein the genetic marker is located in the 5′ UTR of the CAST gene.

6. A method as claimed in claim 5 wherein the allele is CAST5 allele 3.

7. A method as claimed in claim 1 wherein the allele is CAST5 allele 7 or allele 9.

8. A method as claimed in claim 1 wherein the genetic

marker is located in the genomic region embracing the coding sequence of the CAST gene.

9. A method as claimed in claim 1 wherein the genetic marker is a genetic variation located other than in the CAST gene which shows allelic association with either or both of the CAST3 D/E polymorphism and the CAST5 polymorphism.

10. A method as claimed in any one of claims 1 to 9, further comprising the step of testing for the presence or absence of one or more additional genetic markers associated with peak-force variation.

11. A method as claimed in claim 10 further comprising the step of testing for the presence of CAST5 allele 7 or allele 9 and/or the absence of CAST5 allele 3 once the presence of CAST D/E allele 2 has been established.

12. A method as claimed in claim 1 wherein the genetic marker is allele 1 of the LOX polymorphism.

13. A method as claimed in claim 1 wherein the genetic marker is allele 2 of the LOX polymorphism.

14. A genetic marker for meat tenderness in an animal which is a polymorphic form of the CAST gene, being the CAST3 D/E polymorphism or the LOX polymorphism.

15. A genetic marker as claimed in claim 14 wherein the variable portion of the CAST gene comprises the nucleotide sequence set forth in SEQ ID NO:1.

16. A genetic marker as claimed in claim 14 wherein the variable portion of the CAST gene comprises the nucleotide sequence set forth in SEQ ID NO:2.

17. A genetic marker as claimed in claim 14 wherein the variable portion of the CAST gene comprises the nucleotide sequence set forth in SEQ ID NO:3.

18. An isolated DNA molecule comprising the nucleotide sequence set forth in SEQ ID NO:1.

19. An isolated DNA molecule comprising the nucleotide sequence set forth in SEQ ID NO:2.

20. An isolated DNA molecule comprising the nucleotide sequence set forth in SEQ ID NO:3.

21. An isolated DNA molecule consisting of the nucleotide sequence set forth in SEQ ID NO:1.

22. An isolated DNA molecule consisting of the nucleotide sequence set forth in SEQ ID NO:2.

23. An isolated DNA molecule consisting of the nucleotide sequence set forth in SEQ ID NO:3.

24. A method for selecting an animal likely to yield meat of improved tenderness, comprising the steps of:

(1) testing the animal for the presence of an allele of the gene encoding calpastatin (CAST) associated with low peak-force or genetic variation located other than in the CAST gene which shows allelic association with the CAST allele, and/or for the presence of an allele of the LOX gene associated with low instron compression of the semitendinosis muscle or genetic variation located other than in the LOX gene which shows allelic association with the LOX allele; and

(2) selecting animals which have the CAST and/or LOX

allele and/or genetic variation in allelic association therewith.

25. A method as claimed in claim 24 wherein the allele tested for is CAST3 D/E allele 2 and animals which are homozygous for this allele are selected.

26. A method as claimed in claim 25 further comprising the step of testing for the presence or absence of one or more additional alleles of the gene encoding calpastatin associated with low peak-force.

27. A method as claimed in claim 26 wherein the presence of CAST5 allele 7 or allele 9 is tested for, and animals having either of these alleles in addition to CAST3 D/E allele 2 are selected.

28. A method as claimed in claim 26 wherein the presence of CAST5 allele 3 is tested for, and animals having this allele are rejected despite the presence of CAST3 D/E allele 2.

29. A method as claimed in any one of claims 24 to 27, further comprising using the selected animal for breeding.

30. An oligonucleotide probe for amplification of a genetic marker associated with peak-force variation, said genetic marker being either an allele of the gene encoding calpastatin (CAST) or genetic variation located other than in the CAST gene which shows allelic association with said allele.

31. An oligonucleotide probe as claimed in claim 30 selected from the group consisting of:

castd 5′ cat ttg gaa aac gat gcc tca c 3′

caste 5′ tct acg att agc agc tca aga gga g 3′

CAST5U1 5′-GTAAAGCCGCACAAAACACACCCAGG-3′

CAST5D1 5'-GTTTCTGGACCCTCTGGATGAGGAAGCGG-3'

32. An oligonucleotide probe for amplification of a genetic marker associated with variation in instron compression of the semitendinosis muscle, the genetic marker being either an allele of the gene encoding lysyl oxidase (LOX) or genetic variation located other than in the LOX gene which shows allelic association with said allele.

33. An oligonucleotide probe as claimed in claim 31 selected from the group consisting of:

LOX K5: 5' tat cac tga tgt caa acc tg 3'

LOX K6: 5' act cag gca cca aat agc tg 3'

34. A kit for use in assessing the tenderness of meat from an animal and/or selecting an animal likely to yield meat of improved tenderness, comprising oligonucleotide probes for amplification of at least one genetic marker associated with meat tenderness, said genetic marker being either an allele of the gene encoding calpastatin (CAST) or genetic variation located other than in the CAST gene which shows allelic association with said allele, or an allele of the LOX gene associated with low instron compression of the semitendinosis muscle or genetic variation located other than in the LOX gene which shows allelic association with the LOX allele, and means for amplifying DNA.

35. A kit as claimed in claim 34 wherein the oligonucletide probes are selected from the group consisting of:

castd 5' cat ttg gaa aac gat gcc tca c 3'

caste 5' tct acg att agc agc tca aga gga g 3

CAST5U1 5'-GTAAAGCCGCACAAAACACACCCAGG-3'

CAST5D1 5'-GTTTCTGGACCCTCTGGATGAGGAAGCGG-3'

LOX K5: 5' tat cac tga tgt caa acc tg 3'

LOX K6: 5′ act cag gca cca aat agc tg 3′

36. An animal when selected by the method as defined in any one of claims 24 to 27.

37. The progeny of an animal as defined in claim 36.

38. Meat from an animal as defined in claim 36.

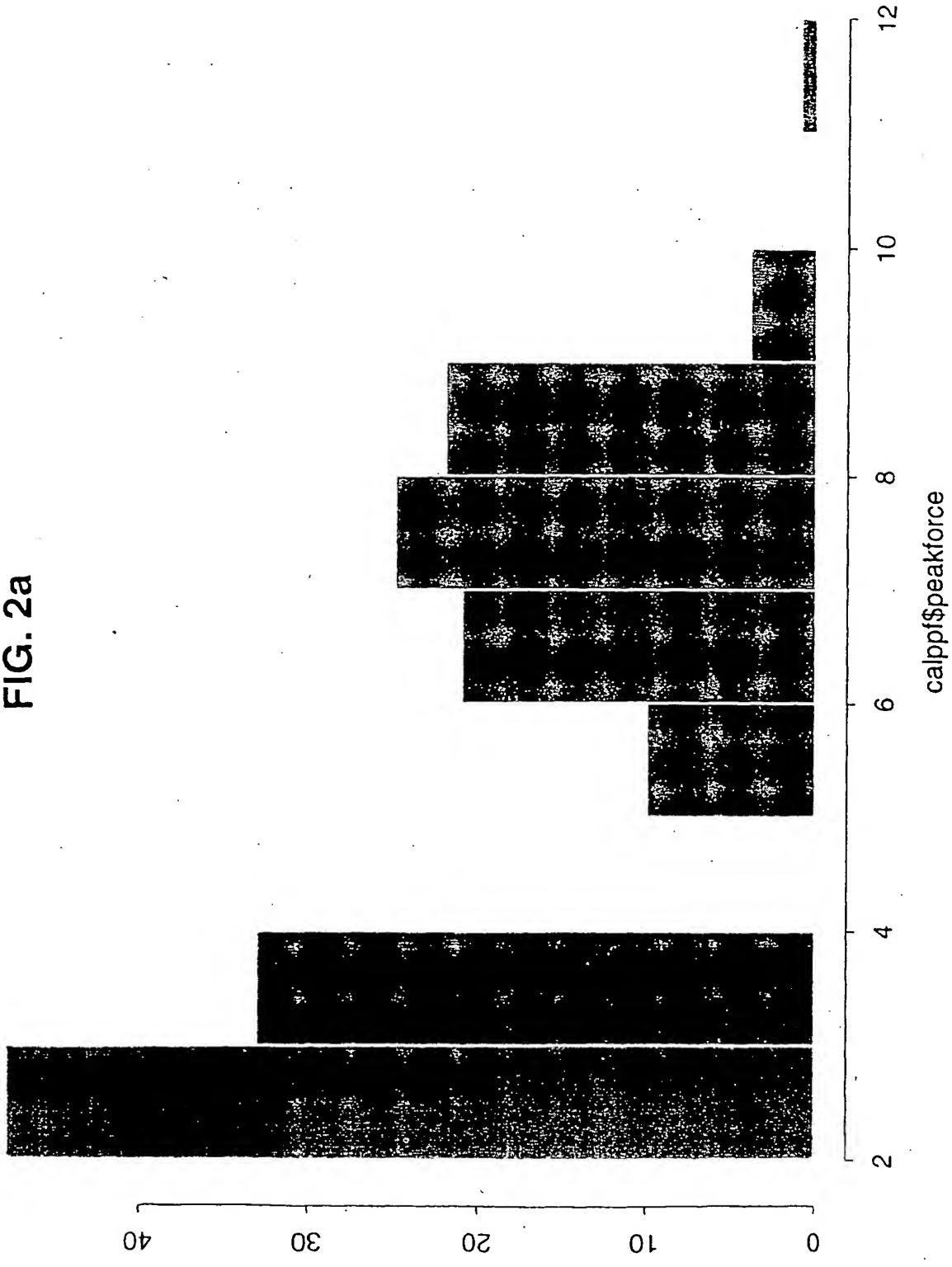
39. Meat from the progeny of an animal as defined in claim 36.

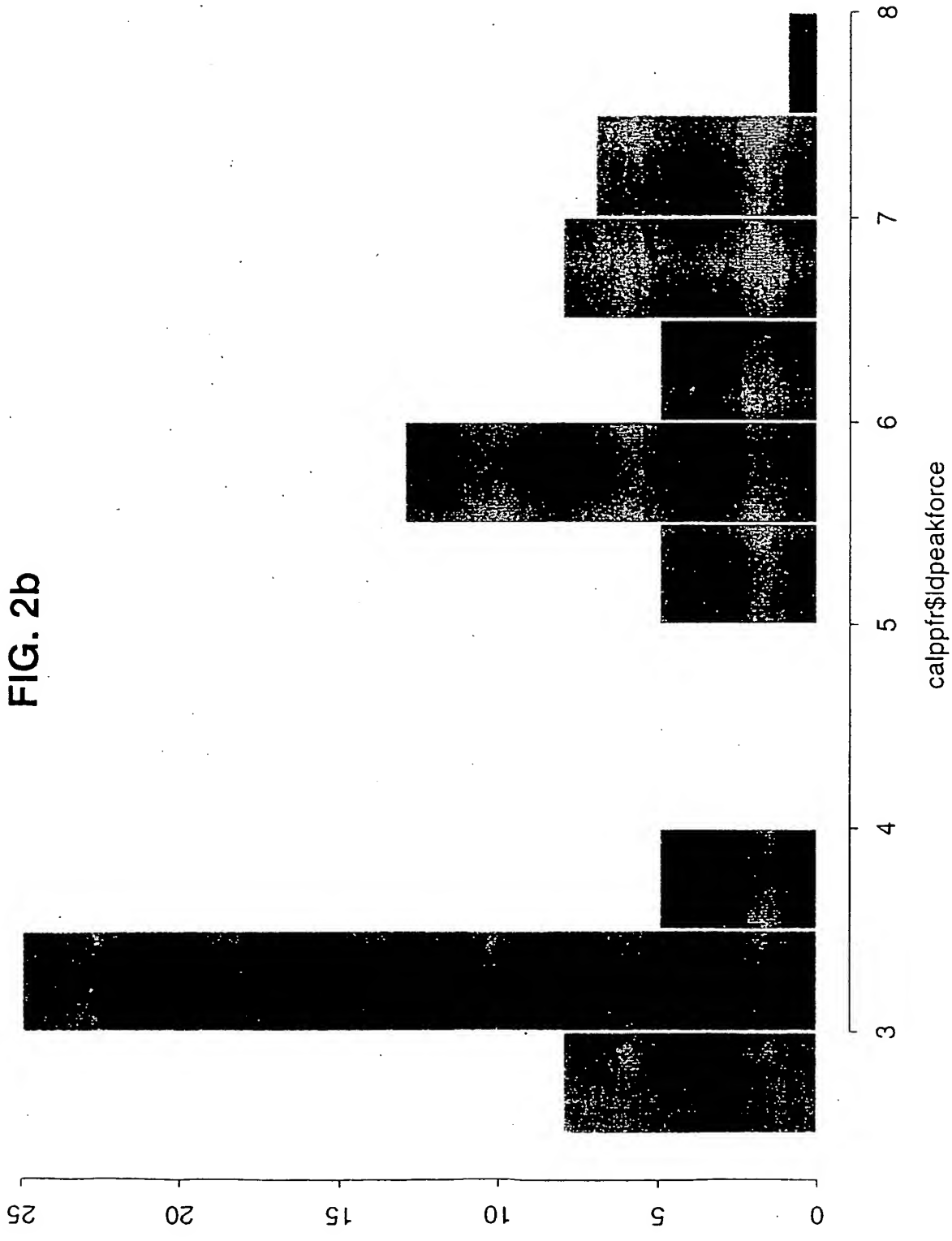
40. The use of an animal as defined in claim 36 in breeding.

41. The use of the progeny of an animal as defined in claim 36 in breeding.

FIG. 1

FIG. 2a





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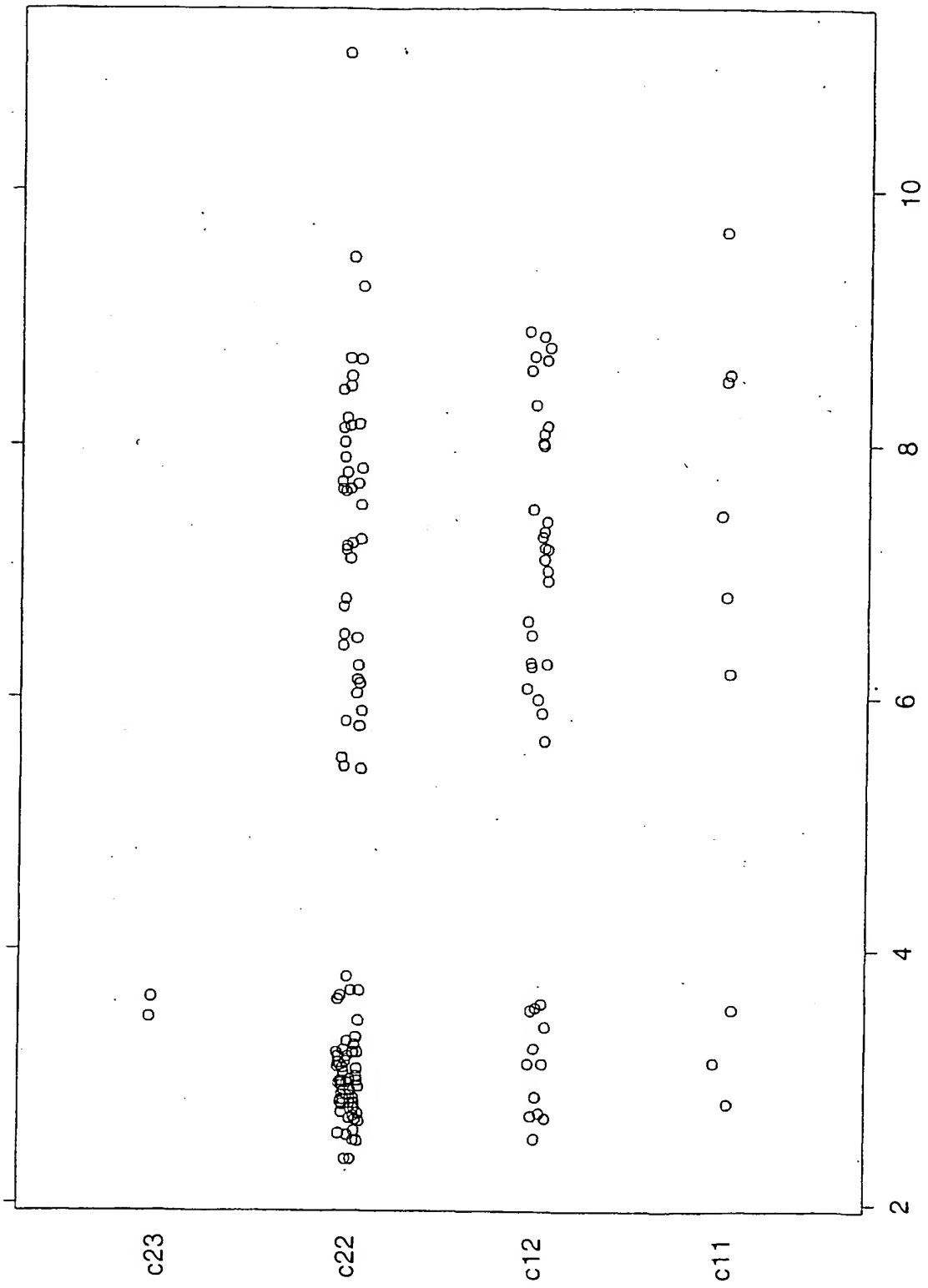

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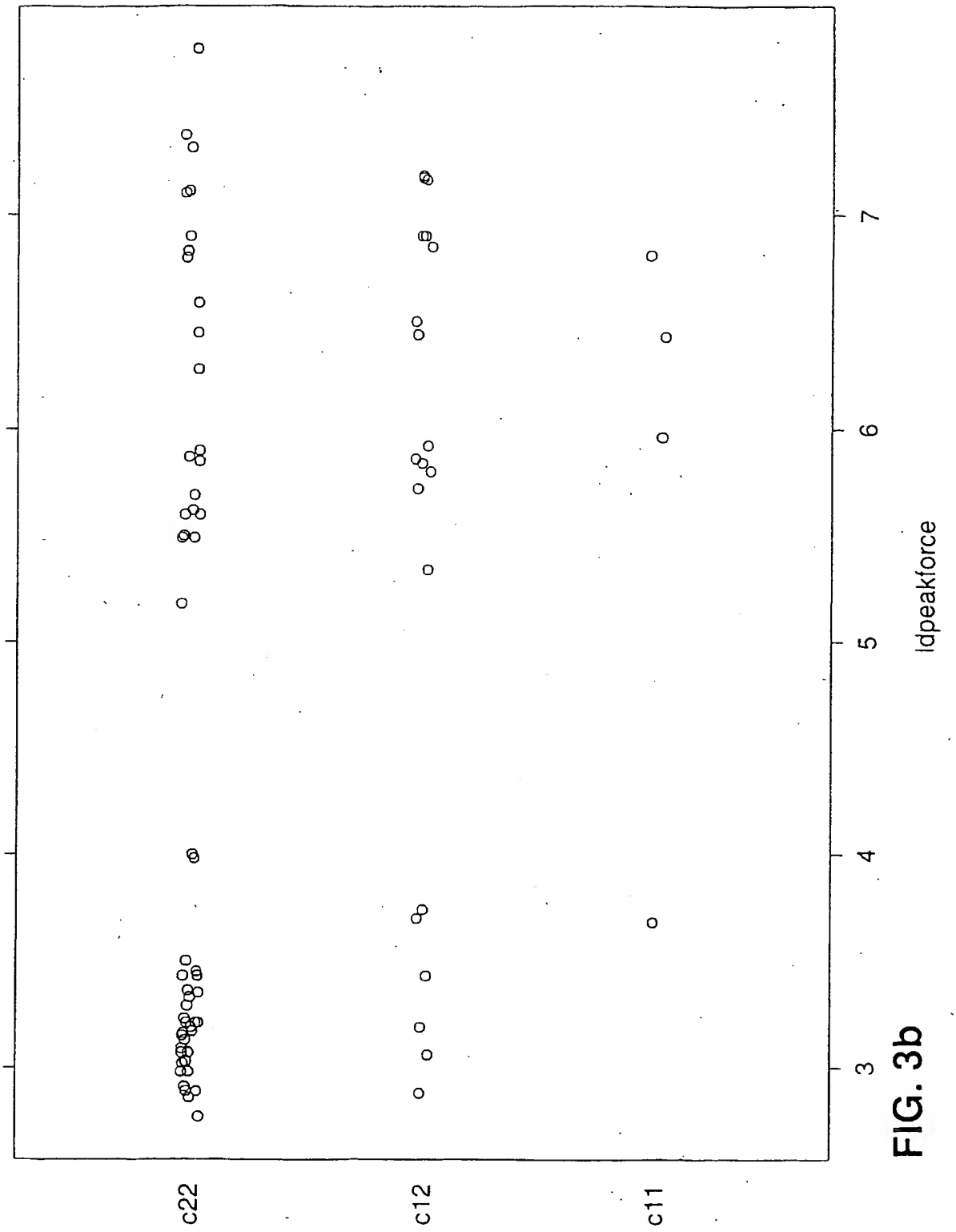

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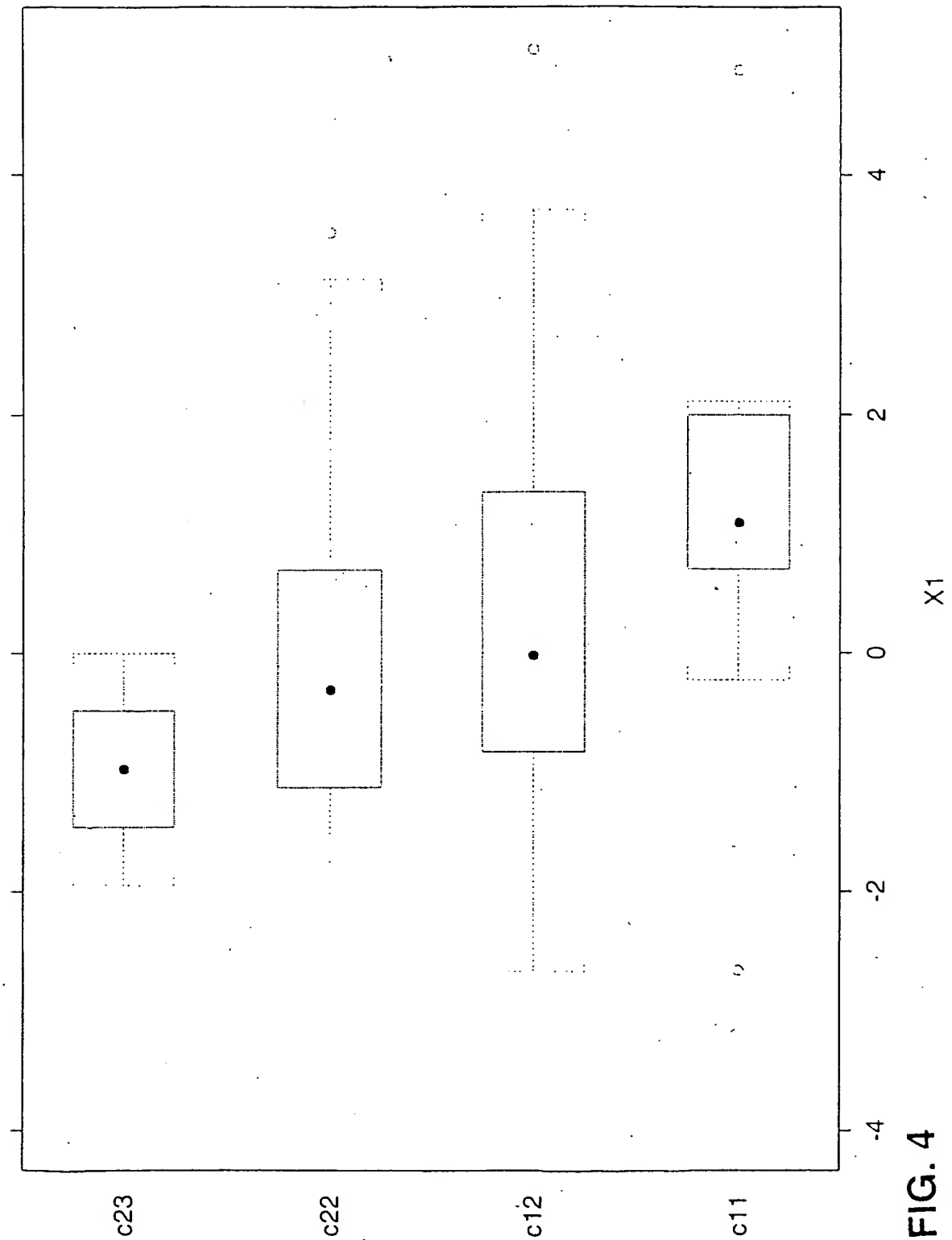

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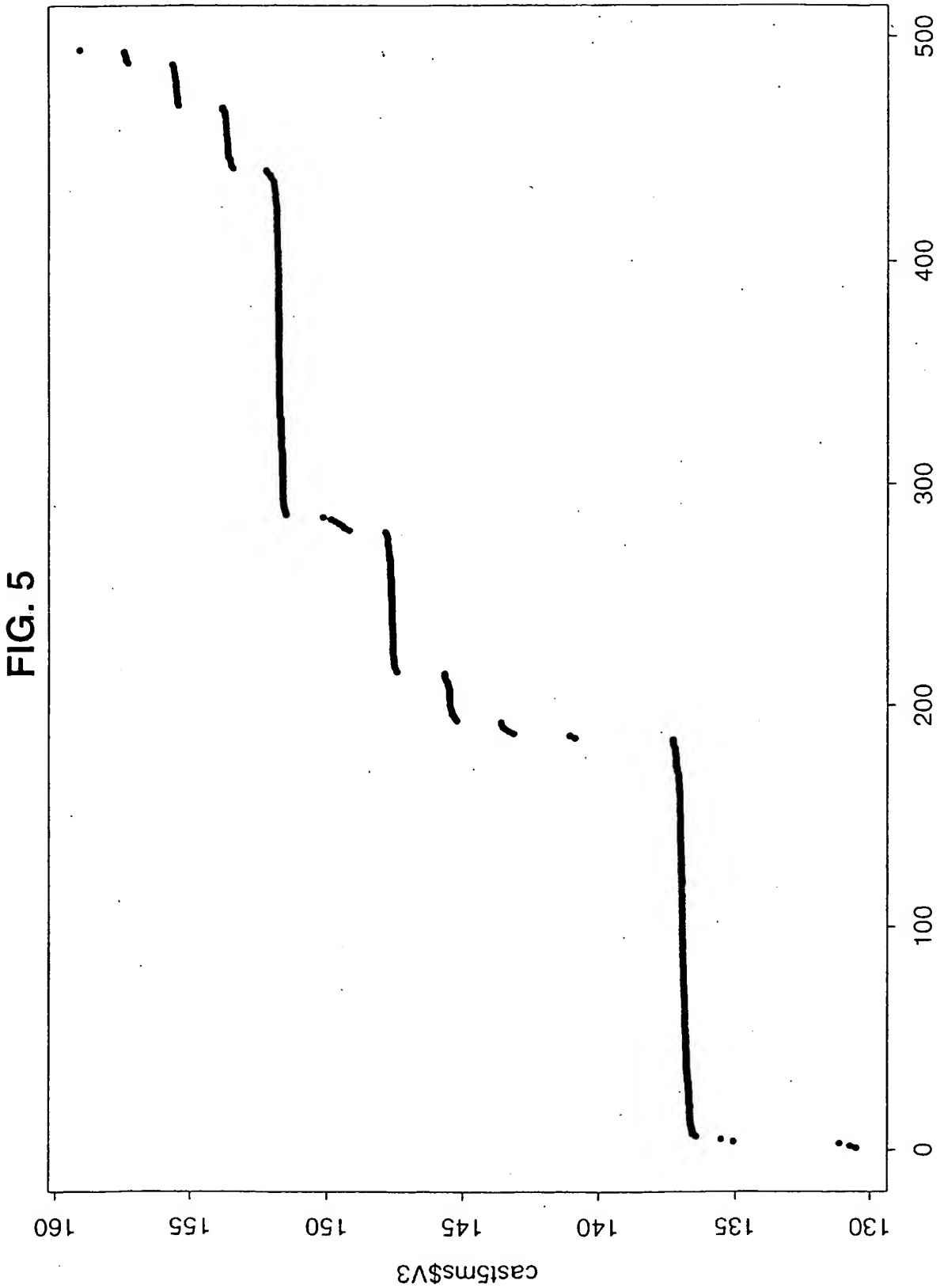

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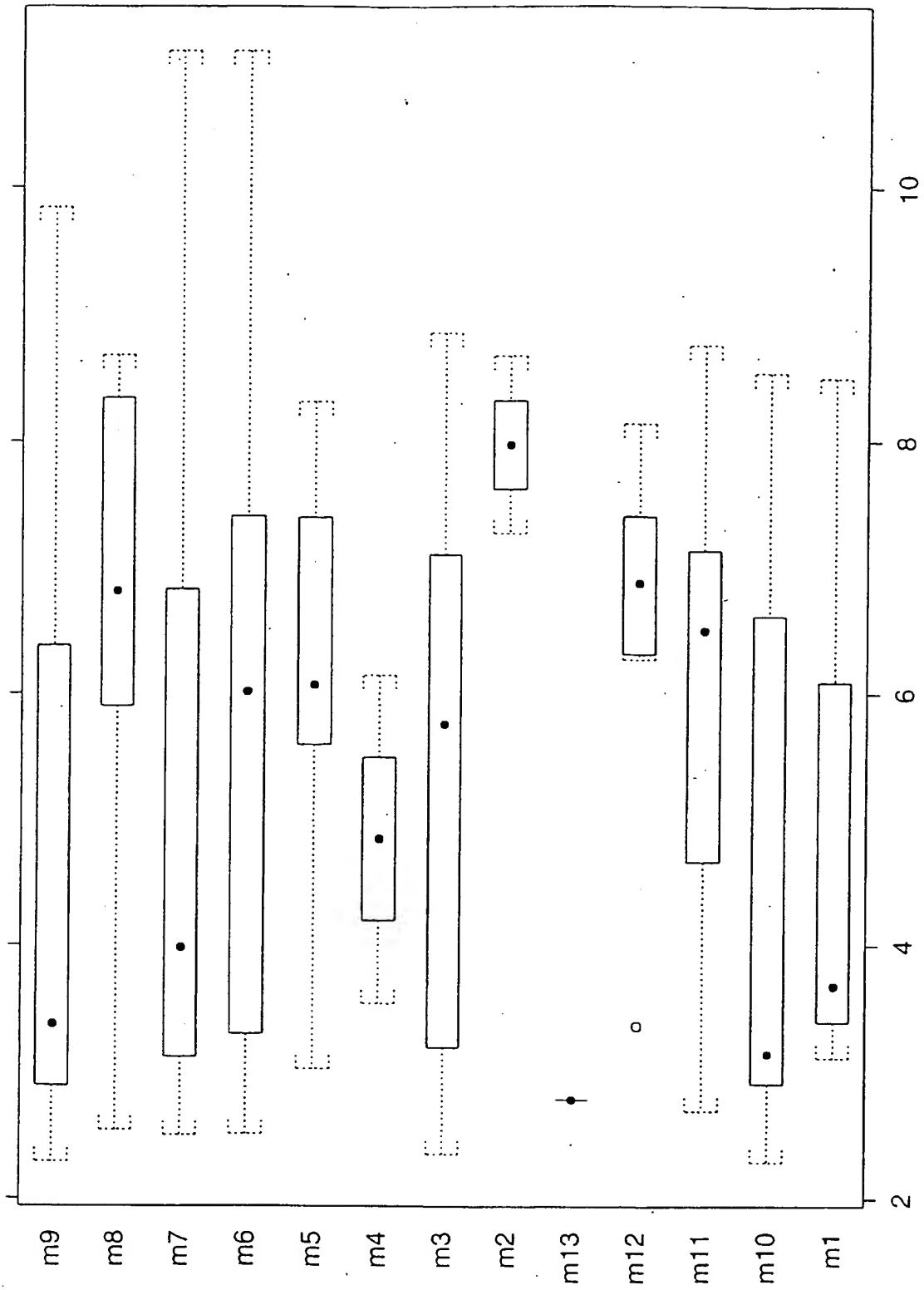

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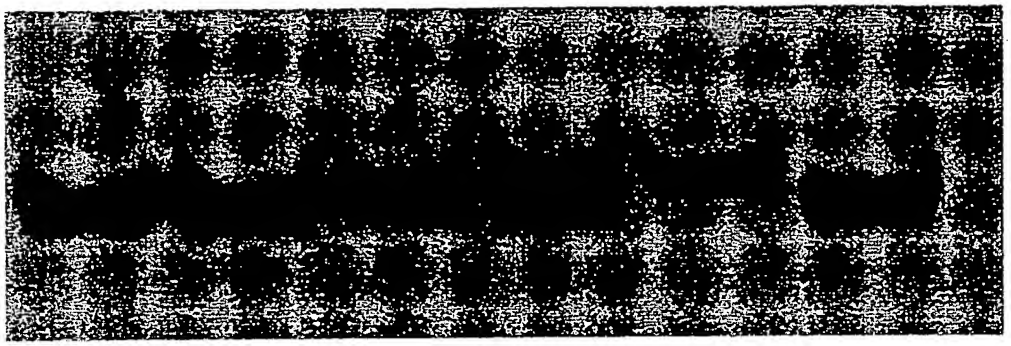


FIG. 7

FIG. 8

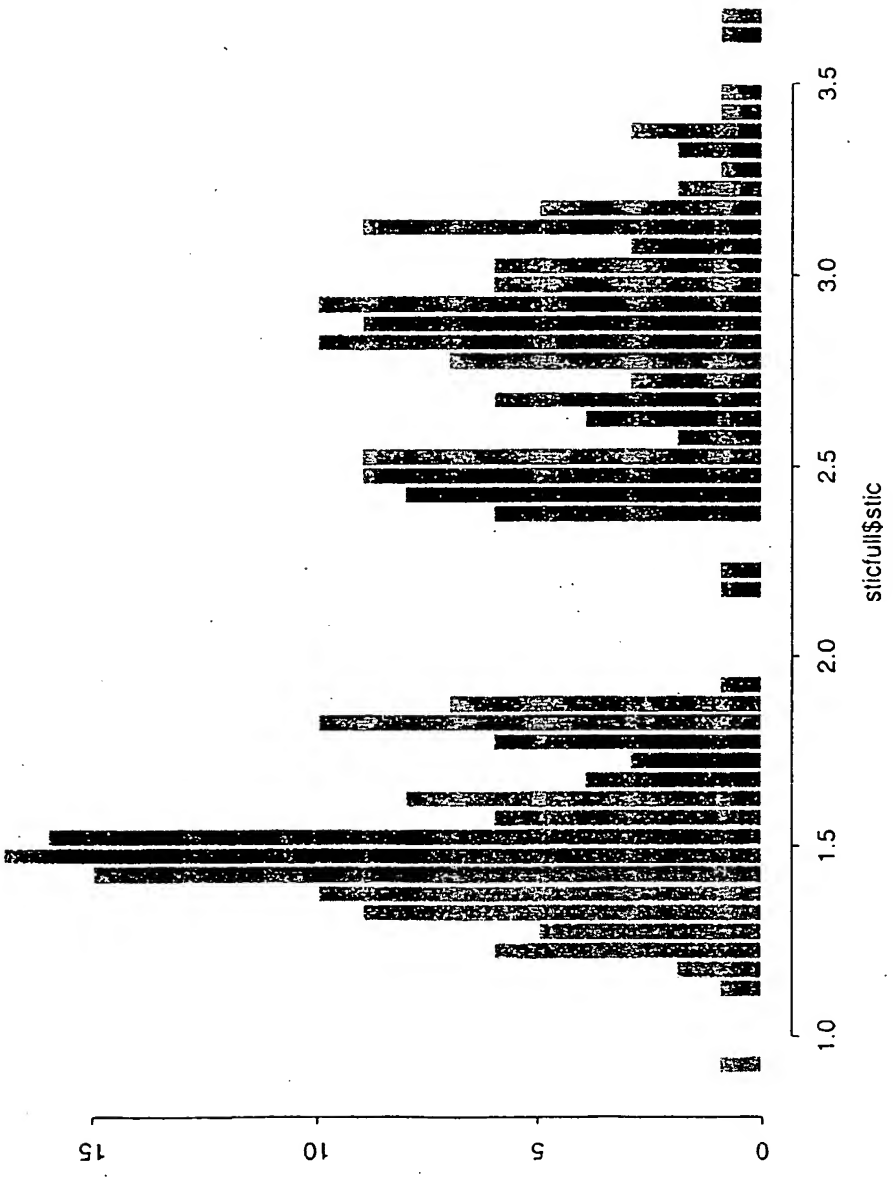


FIG. 9

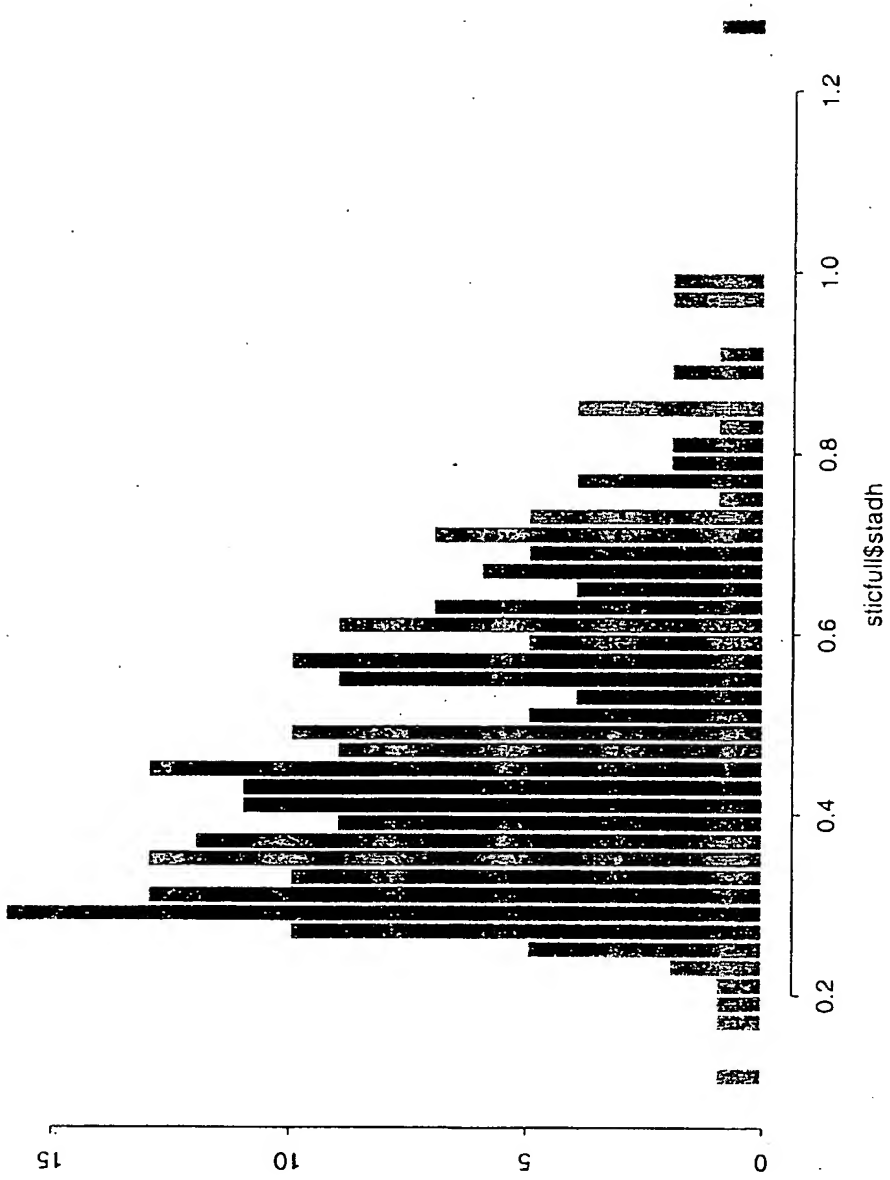
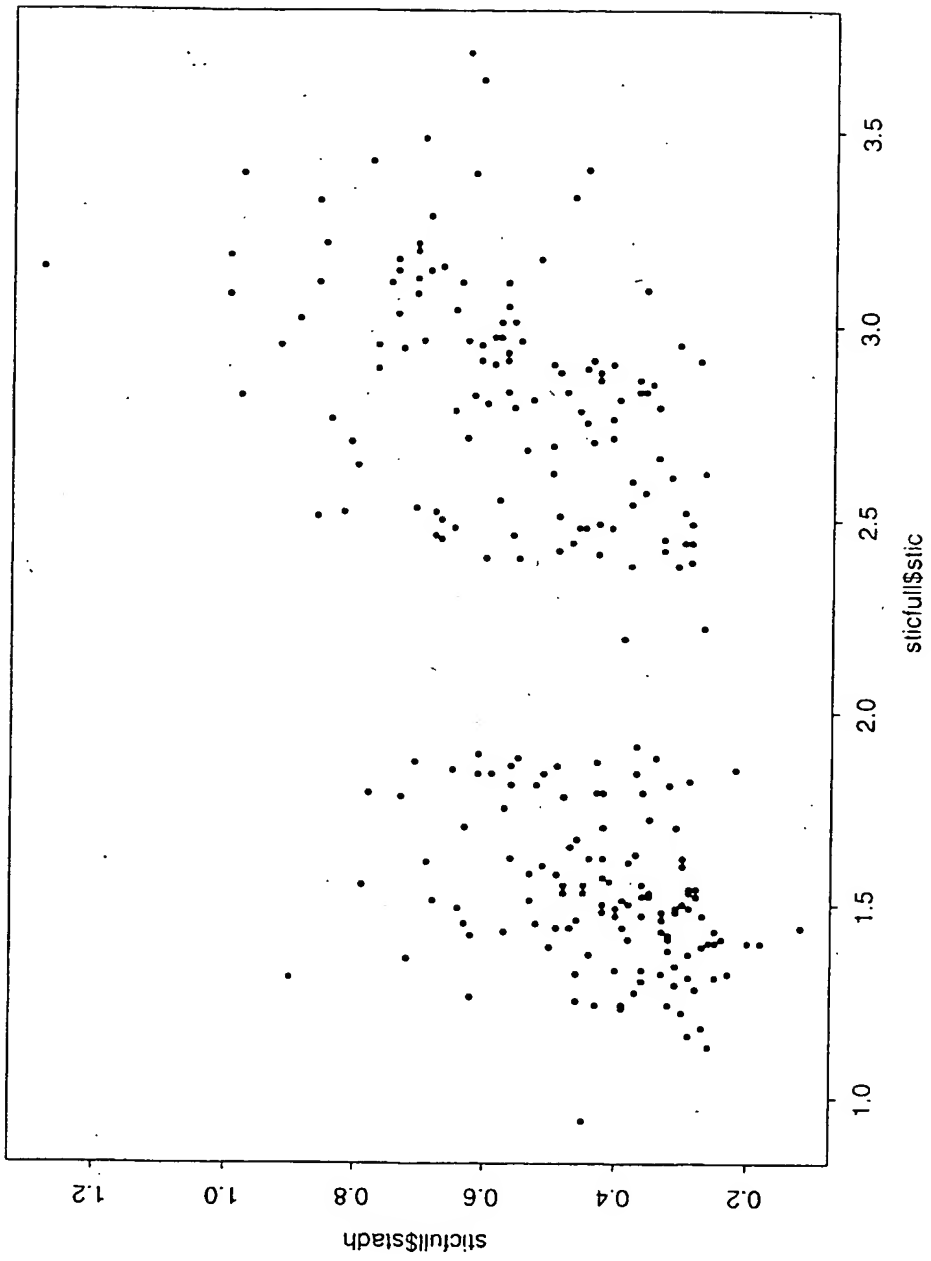


FIG. 10



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The State of Queensland
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Meat and Livestock Australia Limited
The University of New England

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170

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU02/00122

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: C12Q 1/68, C12N 15/11

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Electronic Data Base

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Electronic Data Base

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CA, WPIDS, Medline, Biosis: calpastatin, CAST, polymorphic marker, Lysal oxidase, allele[
ANGIS: Sequence IDs 1 -3

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHUNG, H. Y. et al., "Genetic variants detected by PCR-RFLP in intron 6 of the bovine calpastatin gene", ANIMAL GENETICS, (Feb 2001) Vol 32 (1):53	1-8, 30, 31
X	PALMER,B.R et al., "Single nucleotide polymorphisms in an intron of the ovine calpastatin gene". ANIMAL BIOTECHNOLOGY, (2000) Vol 11 (1) :63-7	1-9, 30, 31
X	PALMER,B.R et al., "A candidate gene approach to animal quality traits". Proceedings of the New Zealand Society of Animal Production, (1997) Vol 57: 294-296.	1-8, 14, 24, 25, 31, 36, 38, 39

[X] Further documents are listed in the continuation of Box C [ ] See patent family annex

* Special categories of cited documents:

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
12 April 2002

Date of mailing of the international search report
18 APR 2002

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU02/00122

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GREEN, R. D. et al, " Association of a Tacq1 calpastatin polymorphism with postmortem measures of beef tenderness in Charolais- and Limousin-sired steers and heifers". Journal of Animal Science, (1996) Vol 74, No. SUPPL. 1 : 113.	1-8, 14, 24, 25, 31, 36-41
X	GREEN, R. D. et al, "Association of a Taq1 calpastatin polymorphism with postmortem measures of beef tenderness in Bos taurus and Bos indicus-Bos taurus steers and heifers". Journal of Animal Science, (1996) Vol. 74, No. SUPPL. 1: 111.	1-8, 30
X	LONERGAN, S. M. et al, "Relationship of restriction fragment length polymorphisms in the bovine calpastatin gene to muscle calpastatin activities and meat tenderness". Journal of Animal Science, (1995) Vol. 73, No. SUPPL. 1: 62.	1-8, 14, 24, 25, 30, 31, 36-41
A	LONERGAN, S. M, "Relationship of restriction fragment length olymorphisms (RFLP) at the bovine calpastatin locus to calpastatin activity and meat tenderness". Journal of Animal Sciences". (1995 Dec) 73 (12): 3608-12.	
A	CHUNG, H. Y. et al., "A DNA polymorphism of the bovine calpastatin gene detected by SSCP". ANIMAL GENETICS, (1999 Feb) 30 (1) 80.	